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2. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey *48*:1 (Jan.) 1951.
3. Shapiro, S., et al.: Am. Heart J. *40*:766 (Nov.) 1950.

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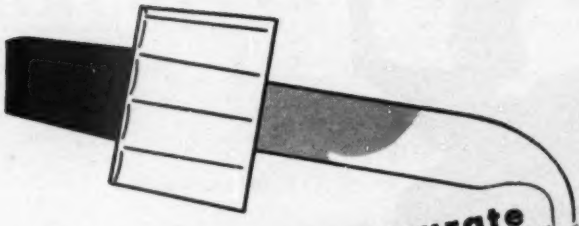

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PEROSIS

Epiphyseal Cartilage in Choline and Manganese Deficiencies in the Chick

S. BURT WOLBACH, M.D.
AND
D. MARK HEGSTED, Ph.D.
BOSTON

INTEREST in all conditions affecting endochondral growth of bone prompted this study of perosis or "slipped tendon" disease of chicks. The purpose of this report is to describe the effects upon the epiphyseal cartilages of young chicks produced by diets deficient in either choline or manganese and deficient in the two factors.

The most conspicuous signs of these deficient diets are enlargement, deformity, and disability of the hock joint, i. e., the articulation of the tibiotarsus and tarsometatarsus bones.

Although much has been written about the dietary regimens, chemistry of bones, and calcium and phosphorus metabolism of the condition in chicks, there is only one good account of the pathology, that of manganese deficiency by A. Levin Nielsen.[1] His pathologic studies are included in a monograph in Danish which covers all aspects of the subject.[2]

Our work confirms A. Levin Nielsen's important conclusion that the epiphyseal cartilage is the seat of the changes responsible for the retardation of growth and the deformities of the condition commonly called perosis or "slipped tendon" disease.

Because, in general, careful pathologic studies have not been made, the results of the dietary deficiencies have often been regarded as peculiar to the hock joint. Even Nielsen [1] restricted his x-rays and histologic studies to the hock joint. All epiphyseal cartilages are affected. We have studied particularly the hock joint and the knee joint, giving preference to the latter, the articulation of the femur with the tibiotarsus, because it is anatomically simpler than the hock joint owing to the fact that at the growth period dealt with in the first weeks of life the unions of the

This study was aided by research grants from The Williams-Waterman Fund for the Combat of Dietary Disease, The Nutrition Foundation, and Swift and Company, Chicago.

From the Division of Nutritional Research, Division of Laboratories and Research, the Children's Hospital; the Department of Nutrition, Harvard School of Public Health, and the Department of Biological Chemistry, Harvard Medical School.

1. Nielsen, A. L.: Perosis: En knoglesygdom hos høns, Nyt Nordisk Forlag, Copenhagen, Arnold Busck, 1942.

2. Unfortunately this important publication has not been widely distributed in this country, although a short abstract was published in Biological Sciences **18**:1, 1944. The only copy of the monograph that we could locate was in the library of the New York Academy of Sciences. The bibliography up to and including 1940 is quite complete.

tarsal bones with the tibia and metatarsus are incomplete. Nielsen repeatedly described the tarsal bones as epiphyses of the tibia and metatarsus but without invalidating in any respect his conclusions.

The gross anatomical changes of perosis result from defective growth sequences of the epiphyseal cartilages, including matrix formation. The consequences are shorter and thicker bones, bowing of the distal tibiotarsus and proximal tarsometatarsal ends, rotation of the latter, with loss of alignment with the tibiotarsus. The articular cartilages become displaced and the tendon of the gastrocnemius muscle becomes dislodged from the intercondyloid groove so that the chick is unable to straighten the leg, and the hock joint becomes directly weight-bearing and permanently deformed, with trauma an added factor.

Other dietary deficiencies which have been reported as productive of hock joint abnormality grossly similar to that of perosis include biotin,[3] nicotinic acid,[4] and riboflavin.[5] The feeding of high levels of thiouracil (0.5%) to young chicks over a period of five weeks has been reported as resulting in "an enlargement of the tibiometatarsal joint and a thickening and bending of the metatarsal," which were not prevented by the addition to the diet of manganese, choline, biotin, nicotinic acid, or riboflavin. Presumably the results obtained were in consequence of thyroxine deficiency.[6]

The possibility that all of these deficiencies during skeletal growth have identical consequences upon epiphyseal cartilage has important implications. It would imply that all of these agents or their appropriate enzyme systems are involved in the metabolic chain of events necessary for the epiphyseal cartilage sequences required for normal bone growth and, more remarkable, that regardless of the particular link removed from this chain of events the end results on endochondral bone growth should be the same. If such is true, it has no counterpart in nutrition experience (with possible exception of simple retardation of growth common to many deficiencies) since, in general, the pathologic end results in cells and tissues of single nutritional deficiencies are specific and distinctive. We regard it as highly desirable and even necessary that perosis should be characterized more definitely than can be done by gross observation. The literature on perosis is mainly American. A report in an American journal in corroboration and extension of Nielsen's limited but informative account of the pathology of perosis is highly desirable.

EXPERIMENTAL

Three groups of chicks were used. All were placed on the experimental diets at 4 days of age. The control diet for Group 1 had the following composition. The low-choline diet had the choline omitted. The low-manganese diet had manganese omitted from the salt mixture and

3. Richardson, L. R.; Hogan, A. G., and Miller, O. N.: The Relation of Biotin to Perosis in Chicks, Research Bulletin 343, Columbia, Mo., University of Missouri College of Agriculture Experimental Station, June 3, 1942. Jukes, T. H., and Bird, F. H.: Prevention of Perosis by Biotin, Proc. Soc. Exper. Biol. & Med. **49**:231, 1942.

4. Briggs, G. M., Jr.; Luckey, T. D.; Tepley, L. J.; Elvehjem, C. A., and Hart, E. B.: Studies on Nicotinic Acid Deficiency in the Chick, J. Biol. Chem. **148**:517, 1943.

5. Bird, F. H.; Asmundsen, V. S.; Kratzer, F. H., and Lepkovsky, S.: The Comparative Requirements of Chicks and Turkey Poults for Riboflavin, Poultry Sc. **25**:47, 1946.

6. Briggs, G. M., and Lillie, R. J.: Perosis Caused by Feeding High Levels of Thiouracil, Proc. Soc. Exper. Biol. & Med. **61**:430, 1946.

2% $CaHPO_4$ added. High-calcium diets apparently interfere with manganese absorption and thus assist in producing a manganese deficiency.[7] The control diet for Group 1 chicks was as follows:

	Per 100 Gm.	Vitamins	
Sucrose	58 gm.	Thiamine chloride	400 γ
Gelatin	10 gm.	Riboflavin	800 γ
Casein	18 gm.	Pyridoxine hydrochloride	400 γ
Corn oil	5 gm.	Nicotinic acid	3,000 γ
Cod liver oil	1 gm.	Alpha tocopherol	10 mg.
Salts IV [8]	5 gm.	Menadione	200 γ
$CaHPO_4$	1 gm.		
Alcohol-extracted liver fraction "L" (Wilson)	2 gm.		
Alcohol-extracted liver fraction (Wilson)	3 gm.		
Calcium gluconate	2 gm.		
Choline chloride	0.3 gm.		

The diet for chicks of Groups 2 and 3, deficient both in manganese and choline, was as follows:

	Per 100 Gm.	Vitamins	
Glucose	56.5 gm.	Thiamine chloride	400 γ
Casein	20.0 gm.	Riboflavin	800 γ
Gelatin	8.0 gm.	Pyridoxine hydrochloride	400 γ
Corn oil	5.0 gm.	Calcium pantothenate	2,500 γ
Cellufiour	3.0 gm.	Nicotinic acid	4,000 γ
$CaHPO_4$	1.0 gm.	Folic acid	100 γ
Cod liver oil	1.0 gm.	Biotin	20 γ
Salts IV minus $MnSO_4$	5.0 gm.	Menadione	400 γ
		Alpha tocopherol	5 mg.

PATHOLOGIC HISTOLOGY

In a previous paper [9] we have described in considerable detail the endochondral growth of bone in the chick (Fig. 1). Our purpose now is to describe the effects of manganese and choline deficiencies and the deficiency of the two upon epiphyseal cartilages in explanation of the outstanding gross characteristics of perosis, retardation of growth of the long bones, deformity of the hock joint, "slipped tendon," and bowing of the ends of the bones of this articulation.[10] As described by Nielsen [1] for manganese deficiency, the explanation resides in disturbed epiphyseal cartilage sequences which result in defective endochondral bone growth.

A few chicks not included in the Tables were placed upon the deficient diets at 4 days of age for a period of 14 days. Three maintained on the manganese-deficient diet and three maintained on the choline-deficient diet were studied histologically. Comparisons with chicks kept for longer periods on the deficient dietary regimens

7. Perosis, Nutrition Rev. **2**:50, 1944.

8. Hegsted, D. M.; Mills, R. C.; Elvehjem, C. A., and Hart, E. B.: Choline Deficiency in Chicks, J. Biol. Chem. **138**:459, 1941.

9. Wolbach, S. B., and Hegsted, D. M.: Endochondral Bone Growth in the Chick, A. M. A. Arch. Path. **54**:1, 1952.

10. (*a*) Caskey, C. D.; Gallup, W. D., and Norris, L. C.: The Need for Manganese in the Bone Development of the Chick, J. Nutrition **17**:407, 1939. (*b*) Jukes, T. H.: Effect of Choline and Other Supplements on Perosis, ibid. **20**:445, 1940. (*c*) Hogan, A. G.; Richardson, L. R.; Patrick, H., and Kempster, H. L.: Perosis Due to a Vitamin Deficiency, ibid. **21**:327, 1941. (*d*) Nielsen.[1]

showed that the distinctive changes in the epiphyseal cartilages were well established by the 14th day and that they were more advanced in the choline-deficient chicks than in the manganese-deficient chicks.

From the latter it was evident that the early changes in epiphyseal cartilage sequences were in the zone of enlarging cells and in the zone of maturing cells.[9] The

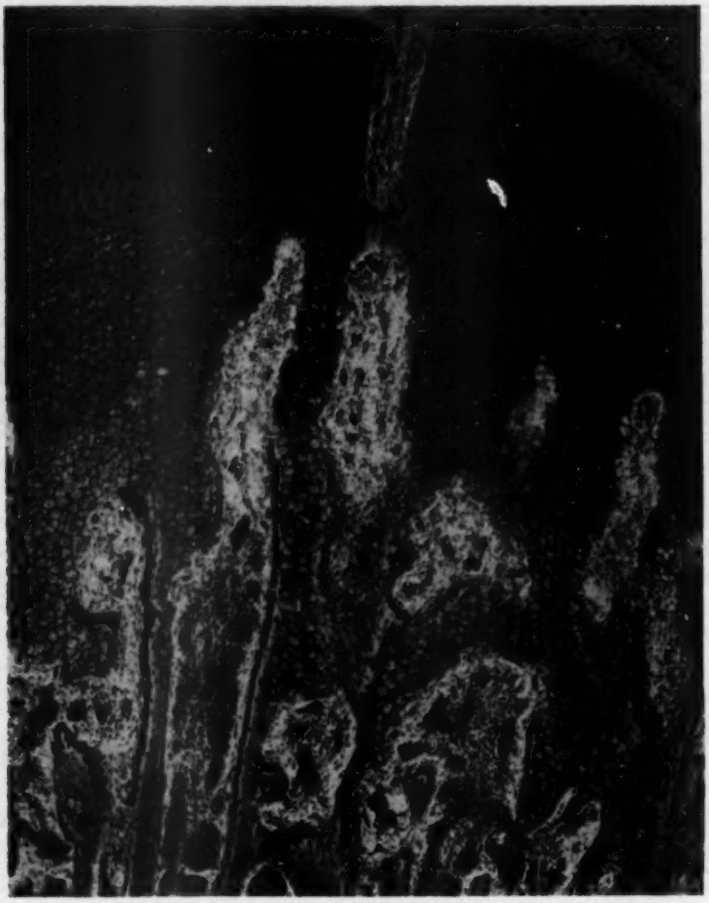

Fig. 1.—Proximal epiphyseal cartilage of a chick on a normal diet and killed after 19 days. This chick was a control to choline-deficient Chick 173, listed in Table 1. Compare with photomicrographs of Chick 173 in Figures 6, 7, and 8. The epiphyseal cartilage is that of the distal end of the tibia. At the age of this chick the ossification of the tarsal bones is incomplete, and they have not fused with the tibia; × 92.

zone of flattened proliferating cells was in one instance wholly normal; in the other two there was slight increase in the matrix. After 14 days on experiment, all epiphyseal cartilage zones were involved in the choline-deficient chicks, and in both manganese- and choline-deficient chicks there was retarded tunneling of the epi-

physeal cartilage and abnormal cytology of maturing cartilage cells. The details of these changes, which will be described for experiments of longer duration, were always more pronounced in the choline-deficient chicks. The smaller effects of the manganese deficiency are presumably the result of small amounts of manganese in the diet or body stores of manganese so that the deficiency was less severe than that produced for choline. The differences do not imply that choline is necessarily more important for the maintenance of epiphyseal cartilage metabolism.

The following description is based upon the study of the epiphyseal cartilages of the knee and hock joints of all of the chicks listed in Tables 1, 2, and 3. Because

TABLE 1.—*Group 1 Chicks* *

Chick	Diet	Initial Weight, Gm.	Final Weight, Gm.	Weight Gain, Gm.	Degree of Perosis	
					Histologic	Gross †
137	Choline-deficient	42	181	139	++++	++
178	Choline-deficient	37	171	134	++++	++
120	Choline-deficient	36	141	105	+++	+
121	Choline-deficient	34	113	79	+++	++
204	Mn-deficient	33	220	187	+++	+±
300	Mn-deficient	37	200	163	+++	+++
209	Control	31	243	212	0	0
245	Control	39	298	259	0	0
173 ‡	Choline-deficient	38	77	39	++++	0

* All chicks of Group 1 were kept on the dietary regimen for 36 days, except Chick 173, which was killed after 19 days.
† Estimated upon deformity and disability of the hock joint.
‡ This chick was killed after 19 days on the deficient diet.

TABLE 2.—*Group 2 Chicks* *

Chick	Diet	Initial Weight, Gm.	Final Weight, Gm.	Weight Gain, Gm.	Degree of Perosis †	
					Histologic	Gross
13	No choline, no Mn	48	48	0	++++	0
311	No choline, no Mn	53	74	21	+++±	0
382	No choline, no Mn	47	59	12	++++	+
43	0.1% Mn, 0.3% choline	47	148	101	0	0
315	0.1% Mn, 0.3% choline	52	207	155	0	0
373	0.1% Mn, 0.3% choline	47	99	52	0	0

* All chicks of Group 2 were kept on the dietary regimen for 18 days.
† Represents an attempt to estimate disability; ++++, both for histologic evidence and for disability indicates the maximum degrees encountered by us.

the differences present in the epiphyseal cartilages in the experiments of 18-, 23-, and 36-day durations were not pronounced and only quantitative, we shall not consider them separately in our description.

The Tables include our attempt to correlate the histologic findings with the gross effects of the deficient diets. We make no attempt at explanation of the incompatibilities found, but it is apparent that the degree of histologic change can often not be assessed by gross observation.

The least degree of histologic perosis includes failure of maturation of the cartilage cells, retarded tunneling, some abnormal matrix in the zone of growth, and a normal or very slightly changed zone of proliferation. Maximum histologic perosis involves all zones of the epiphyseal cartilage and includes changes in cytology and

matrix of all zones and indications of complete cessation of endochondral growth of bone. Severity of perosis in the chick before sacrifice was estimated by the degree of deformity of the hock joint and functional disability.

The Tables also show the smaller growth of the choline-deficient chicks and those deficient in both choline and manganese.

Because of the complete similarity of findings in epiphyseal cartilage other than quantitative in the two deficiencies, we shall not describe them separately.

At the age periods studied, the proximal two tarsal bones and the distal tarsal bone are incompletely ossified and are respectively separate from the tibia and metatarsus. In general, ossification of the tarsal bone was more advanced in the aged control chicks than in the deficient chicks. Of the latter, perhaps as a consequence of greater growth retardation, ossification of the tarsal bones was somewhat less advanced in the choline-deficient chicks. We have been unable to find a statement of the time at which the tarsal bones fuse with the tibia and metatarsus, but the role

TABLE 3.—*Group 3 Chicks* *

Chick	Diet	Initial Weight, Gm.	Final Weight, Gm.	Weight Gain, Gm.	Degree of Perosis †	
					Histologic	Gross
343 ‡	50 γ Mn, 1 mg. choline	51	105	54	+++±	++±
374 ‡	50 γ Mn, 9 mg. choline	50	157	107	+++±	++
347 ‡	1.35 mg. Mn, 1 mg. choline	54	100	46	+++	+++±
357	1.35 mg. Mn, 27 mg. choline	50	158	108	+±	0
361	No Mn, no choline	50	91	41	+++±	++
352	No Mn, no choline	55	100	45	+++±	+++±
350	1 mg. Mn/gm. diet, no choline	52	70	18	+++±	+±
333	1 mg. Mn/gm. diet, no choline	55	104	49	+++±	+±
339	No Mn, 0.3% choline	53	210	157	+++±	+
330	No Mn, 0.3% choline	55	198	143	+++±	+++
316	1 mg. Mn/gm. diet, 0.3% choline	48	186	138	0	0
385	1 mg. Mn/gm. diet, 0.3% choline	55	215	150	0	0

* All chicks of Group 3 were kept on the dietary regimen for 23 days.
† Represents an attempt to estimate disability; ++++, both for histologic evidence and for disability indicates the maximum degrees encountered by us.
‡ Fed by pipette daily with the amounts indicated in the table.

of the epiphyseal cartilages of tibia and metatarsus in linear growth of the bones and the absence of epiphyseal cartilage in the tarsal bones clearly indicate that union to form the tibiotarsus and tarsometatarsus must take place after completion of growth of the long bones.

The articular cartilages of the manganese- and choline-deficient chicks at all periods studied showed no change. In Chicks 13, 311, and 382, deficient in both manganese and choline, there were slight changes in the zone of proliferation consisting of fewer mitotic figures, more palely stained matrix, and plumper cells than in normal controls and in chicks deficient in either manganese or choline (Figs. 2 and 3). This difference could well be the result of the great retardation of growth produced by the combined deficiency (Table 2).

The epiphyseal cartilages are profoundly changed. Common to manganese deficiency, choline deficiency, and deficiency in these two factors are, apparently in order of sequence, (*a*) failure of cartilage cells to mature, (*b*) presence of an excess of atypical matrix in the zone of growth, and (*c*) excess of matrix and reduction of mitoses in the zone of proliferation. All are accompanied by cytological

Fig. 2.—Proximal tibia of Chick 382, deficient in both manganese and choline (Table 2). Eighteen days on dietary regimen. Note absence of normal tunneling of the epiphyseal cartilage and defective matrix in the various zones of the cartilage; × 25.

changes. Tunneling of the cartilage, which can proceed only as the cartilage cells mature, is retarded or completely suppressed (Figs. 3 and 4). There is no apparent defect in osteogenesis, and therefore all of the matured cartilage cells of tunnel walls become replaced by bone so that the result is that of almost complete disappearance of the cartilage "columns" characteristic of normal endochondral bone growth in birds (Figs. 2, 3, and 4). The epiphyseal-diaphyseal junction becomes changed from a lengthy structure of cartilage tunnels lined with bone to a narrow zone where penetration of bone has been abruptly thwarted by failure of maturation of the

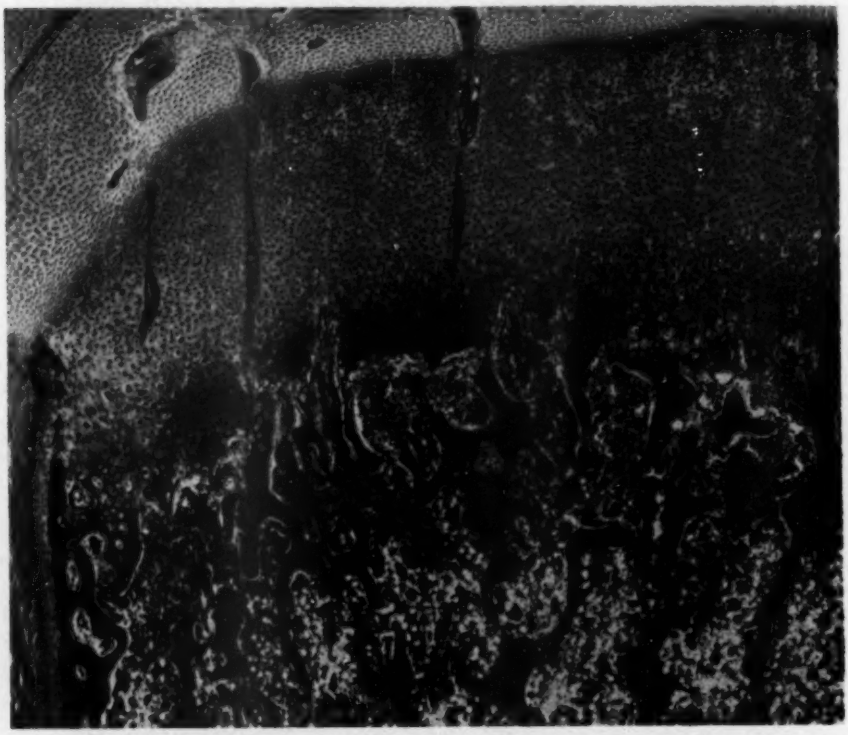

Fig. 3.—High-power detail of Figure 2. Note lightly staining matrix, particularly in the zones of proliferation. Note also the sturdy bone trabeculae and absence of tunneling; × 50.

cartilage cells. This effect, we believe, results in a weak union of epiphyseal cartilage and diaphysis and is the cause of the bowing of the ends of the bones of the hock joint. Distinct bowing of the tibiotarsus and tarsometatarsus was evident in sagittal plane sections of several of the chicks kept for 36 days on the deficient diets. At the sites of the bowing (flexor surface) there was abundant bone of appositional deposition. We could find no evidence that bone deposition, both appositional and wherever epiphyseal cartilage sequences permitted, was affected. We found no evidence that the changes in the epiphyseal cartilage contributed directly to the deformities of the hock joint, although the presence of an increased amount of atypical matrix suggested

participation of the cartilage. Our sections through the hock joint gave no indication of a change in relationship of the incompletely ossified tarsal bones either to the tibia or to the metatarsus.

Zone of Proliferation.—This zone (Fig. 1) normally composed of flat cells transversely arranged to the long axis of the bone, with numerous mitoses and very little matrix,[9] loses its sharp line of demarcation from the zone of growing cells because of an increase in size of cells and of matrix. Increase of size of the cartilage cells and in amount of matrix by the 23rd day may be present in the layer of cells farthest

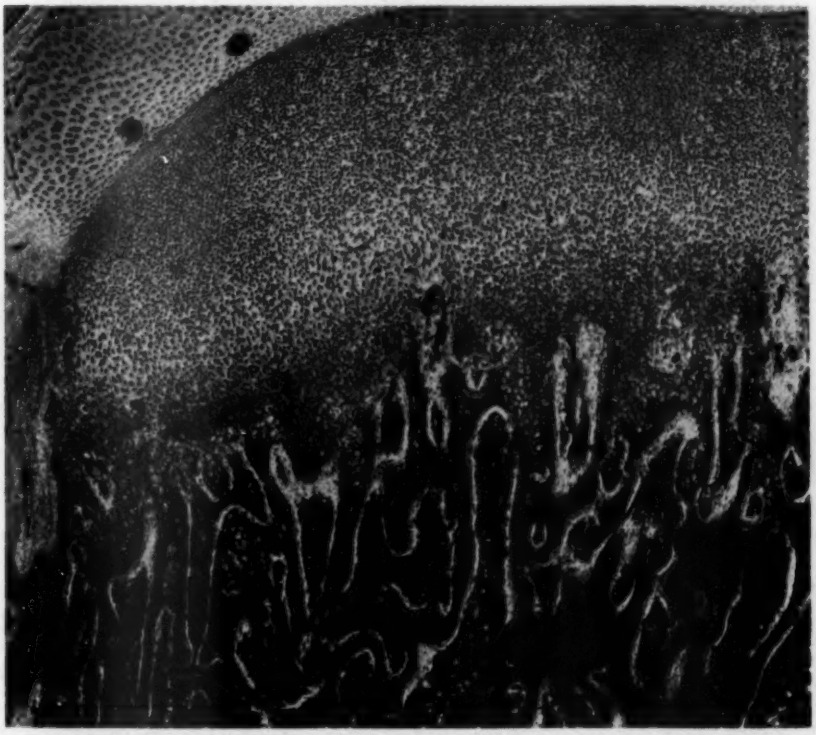

Fig. 4.—Epiphyseal cartilage, proximal tibia of Chick 178 (Table 1). Thirty-six days on choline-deficient diet. Note excessive lightly staining matrix in the zone of proliferation and intermediate zone. Note also defective tunneling and the sturdy bone trabeculae; × 50.

removed from the diaphyseal side (Figs. 3 and 4). The line of demarcation between the epiphyseal cartilages of femur and tibia and articular cartilage may be irregular owing to enlarged epiphyseal cartilage cells surrounded by matrix. The enlargement of the cartilage cells and increase in atypical matrix become progressively greater as the diaphyseal side is approached, and it becomes impossible to demonstrate separation of the epiphyseal cartilage into the zones so easy of identification in normal growing bones. These changes just described were more pronounced in the deficiencies of longest duration. They are more pronounced in choline deficiency (Fig. 4) than in manganese deficiency (Fig. 5) and most pronounced in chicks deficient in

both manganese and choline (Figs. 2 and 3). The same order of change applies to the cartilage cells, which assume a variety of shapes—rounded, ovoid, angulated, and often in extreme instances, as in the combined deficiency, having their longer axis parallel instead of transverse to the long axis of the bone. In choline deficiency and in the combined deficiency there were scattered throughout the epiphyseal cartilage cells which were small, with eosinophilic cytoplasm and densely stained pyknotic nuclei, evidence of occasional death of cartilage cells.

The increased matrix in the choline-deficient chicks and in those with both choline and manganese deficiency stained lightly as compared with normal matrix (Figs. 2, 3, and 4) and with the increased matrix of manganese deficiency (Fig. 5). In the former two deficiencies the matrix most recently formed, as shown by position in the epiphyseal cartilage, is the most lightly stained and in the photomicrographs (Figs. 2 and 3) appears almost to be absent.

Fig. 5.—Epiphyseal cartilage, distal femur of Chick 343. Twenty-three days on manganese-deficient diet. Note the excess of deeply staining matrix and retarded tunneling; × 120.

Intermediate Zone.—Because separation into zones is impossible we prefer to call the presumable zone of growth the intermediate zone. There is little additional to describe in this zone. In the choline-deficient chicks there were small pools of granular matrix, several times in size the dimensions of matured cartilage cells (Fig. 6). In the manganese-deficient chicks the excess matrix stained more deeply with hema-

toxylin than matrix of normal epiphyseal cartilage. This deeply stained matrix did not appear in the form of pools. Cartilage cells, often greatly enlarged and usually with poorly defined capsules, were embedded in it. In greatest excess it appeared in cloud-like form and was distributed throughout most of the epiphyseal cartilage (Fig. 5). Similar deposits of deeply staining excess matrix were also present in the

Fig. 6.—Choline-deficient Chick 173. Thirty-six days on choline-deficient diet. Epiphyseal cartilage, proximal metatarsus. Note the excess of lightly staining matrix and the pools of finely granular accumulation of matrix. Compare with normal control, Figure 1; × 92.

epiphyseal cartilages of Chick 347, fed large amounts of manganese and low choline, and Chick 357, which was fed large amounts of manganese and adequate choline (Fig. 7).

Zone of Maturation.—In all of the epiphyseal cartilages examined, somewhere across the diaphyseal face of the epiphyseal cartilage small groups of fully matured

cartilage cells were present and entered by blood vessels and osteoblasts, but this continuance of normal sequences was not regularly distributed as in normal bones and was overshadowed by the extent of the region of unmatured cells. Nevertheless, continuance of longitudinal growth of the bones was in evidence, more so in manganese deficiency than in choline deficiency and least so in chicks with the combined deficiency.

Cytological changes evident throughout the width of the epiphyseal cartilage were most conspicuous in the zone of maturation. It does not seem advisable to describe

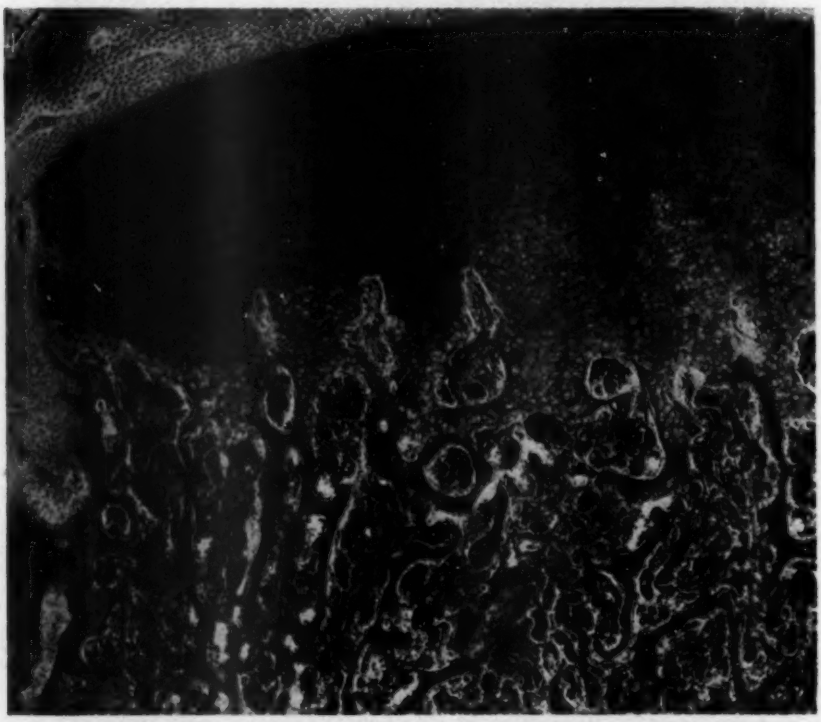

Fig. 7.—Epiphysis, proximal tibia of Chick 347. Maintained 23 days on a dietary regimen supplying 1.35 mg. manganese and 1 mg. choline daily. Note the characteristic findings of perosis, including an excess of basic staining matrix and defective tuneling; × 50.

in detail these changes. Most prominent were abnormal size of the cells, persistence of the cytoplasm, increase in number of nuclei per cell, and densely stained capsules (Figs. 6, 7, and 8). Occasionally regions of very densely stained matrix were present (Fig. 8). The cytological changes were most pronounced in chicks with the choline and combined deficiency (Fig. 9).

Summary of Histopathology.—The essential features of the effects of choline and manganese deficiency upon epiphyseal cartilages are the same and include all sequences involved in endochondral growth of bone, proliferation, growth, matrix formation, and maturation of cartilage cells. Differences in details, particularly in the

characteristics of the excess matrix and in cytological features, may possibly be associated with the greater retardation of growth of the choline-deficient chicks. The differences we have described are sufficiently characteristic and constant to enable us to identify slides of knee or hock joints selected at random as either from choline-deficient or from manganese-deficient chicks. The inference that manganese and

Fig. 8.—High-power detail of Figure 6. Note bone deposition about unmatured cartilage cells, densely staining capsules, and matrix of the cartilage; × 300.

choline meet in a common biochemical system, presumably enzymatic, seems warranted. This is supported by the apparent additive effects obtained by superimposed deficiencies of choline and manganese. Of special interest is the fact that, as indicated in Table 3, two chicks, Nos. 347 and 357, which received amounts of choline and manganese in excess of normal requirements but in disproportion showed all of the histologic changes we have described for chicks in manganese and choline deficiencies (Fig. 7).

The following organs of all the chicks of Table 3 and of Chick 173 (Table 1) were sectioned and studied—heart, lung, spleen, liver, kidney, adrenal gland, pancreas, gizzard, stomach, and intestines. No differences were found in the deficiency as compared with the controls.

Fig. 9.—Chick 173. Distal femur, choline deficiency. Abnormal cytology of nonmaturing cartilage cells; × 300.

COMMENT

In all probability perosis has been produced in turkey poults [11] maintained on choline-deficient diet. The illustrations accompanying Juke's paper [10b] are convincing, but histologic verification would seem desirable. Similarly, the gross abnormalities observed in the hock joints of chicks subjected to various other deficiencies would

11. Enlarged Hock Disorder in Turkeys, Nutrition Rev. **10**:111, 1952. Jukes.[10b]

appear to require histologic study before defined as perosis. This is particularly true since well-defined changes may occur without grossly observable changes in the hock joint.

Whether or not impairment of skeletal growth in mammals, swine,[12] rabbits,[13] rats,[14] and mice [14c] produced by manganese-deficient diets has its origin in epiphyseal cartilage disturbances such as are distinctive for perosis in chicks can only be decided by histologic studies. Wachtel, Elvehjem, and Hart [14b] found no gross signs of skeletal changes in manganese-deficient rats. No histologic studies were made.

Bessey and Wolbach (unpublished experiments) studied the bones of rats which survived the renal lesions resulting from a choline-deficient diet. The rats were placed on the deficient diet at 21 days of age and were studied at periods up to 92 days on the diet. A review of the bones, distal femur and proximal tibia, has shown no departure from the normal other than a slight retardation of endochondral bone growth.

Worthy of note is the fact that the liver cells of our choline- and manganese-deficient chicks did not show the excessive accumulation of fat which occurs in the rat and which has been extensively studied.[15] The large amount of liver fat which is present at hatching is apparently handled normally.[16]

Biochemical studies in both chicks and mammals have been based on the assumption that perosis in chicks and skeletal growth impairment in mammals in manganese deficiency were the result of faults of osteogenesis. We are of the opinion that no biochemical explanation of perosis has been revealed; the epiphyseal cartilages are the logical target for the future studies. The histologic evidence points to normal bone matrix formation and normal calcification in perosis, in both manganese and choline deficiency.

Comparison of the epiphyseal cartilage of perosis with that of other deficiencies is of interest.

In vitamin A deficiency in chicks,[17] ducks,[18] and mammals [19] the epiphyseal cartilages cease all activities—proliferative growth, matrix formation, and maturation. Important changes occur in many epitheliums.[20]

12. Miller, R. C.; Keith, T. B.; McCarty, M. A., and Thorp, W. T. S.: Manganese as a Possible Factor Influencing the Occurrence of Lameness in Pigs, Proc. Soc. Exper. Biol. & Med. **45**:50, 1940.

13. Smith, S. E.; Medlicott, M., and Ellis, G. H.: Manganese Deficiency in the Rabbit, Arch. Biochem. **4**:281, 1944. Ellis, G. H.; Smith, S. E., and Gates, E. M.: Further Studies of Manganese Deficiency in the Rabbit, J. Nutrition **34**:21, 1947.

14. (*a*) Barnes, L. L.; Sperling, G., and Maynard, L. A.: Bone Development in the Albino Rat on a Low Manganese Diet, Proc. Soc. Exper. Biol. & Med. **46**:562, 1941. (*b*) Wachtel, L. W.; Elvehjem, C. A., and Hart, E. B.: Studies on the Physiology of Manganese in the Rat, Am. J. Physiol. **140**:72, 1943. (*c*) Shils, M. E., and McCollum, E. V.: Further Studies on the Symptoms of Manganese Deficiency in the Rat and Mouse, J. Nutrition **26**:1, 1943.

15. Koch-Weser, D.; de la Huerga, J., and Popper, H.: Effect of Choline Supplements on Fatty Metamorphosis and Liver Cell Damage in Choline and Protein Deficiency, J. Nutrition **49**:443, 1953. Shils and McCollum.[14c]

16. Hegsted, D. M.; Mills, R. C.; Elvehjem, C. A., and Hart, E. B.: Choline Deficiency in Chicks, J. Biol. Chem. **138**:459, 1941.

(Footnotes continued on next page)

In vitamin D deficiency (rickets) epiphyseal cartilage sequences continue to be normal in mammals [21] and presumably in chicks [22] in proliferative and growth zones. Matrix formation is normal, but in the zone of maturation the cartilage does not mature, and calcification occurs in neither the cartilage matrix nor the bone matrix, which continues to be formed. No soft tissue changes peculiar to rickets occur.

In vitamin C deficiency in humans and guinea pigs, all supporting tissues are affected by suppression of formation of all intercellular materials.[23] The epiphyseal cartilage cells promptly cease to form matrix and exhibit severe but reversible cytological changes.

In hypervitaminosis A in mammals,[19] chicks,[24] and ducks,[25] there is acceleration of all epiphyseal cartilage cell sequences, which, except for rate of activities, remain normal in all zones. Acceleration of remodeling sequences of bone growth accompany the epiphyseal cartilage behavior. No changes occur in other tissues or organs peculiar to hypervitaminosis A.

The epiphyseal cartilage of perosis, because of defective matrix formation, has a very remote resemblance to the effect of vitamin C deficiency, even though other intercellular materials are not affected. In common with rickets there is failure of maturation of epiphyseal cartilage cells in perosis, but all cartilage cell sequences are affected, and calcification of bone matrix is not suppressed.

The occurrence of the typical epiphyseal cartilage lesions of perosis in the only two chicks, Nos. 347 and 357, of Table 3, which received amounts of manganese in excess of normal requirements, calls for further study and precise determination of the manganese-choline ratio which will elicit the effect (Fig. 7).

CONCLUSIONS

The skeletal manifestations of perosis are the result of retardation or suppression of epiphyseal cartilage sequences, including proliferation of cells, growth of cells, matrix formation, and maturation.

Endochondral bone growth is retarded or suppressed.

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17. Wolbach, S. B., and Hegsted, D. M.: Vitamin A Deficiency in the Chick: Skeletal Growth and the Central Nervous System, A. M. A. Arch. Path. **54**:13, 1952.

18. Wolbach, S. B., and Hegsted, D. M.: Vitamin A Deficiency in the Duck: Skeletal Growth and the Central Nervous System, A. M. A. Arch. Path. **54**:548, 1952.

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21. Wolbach.[19] Wolbach and Bessey.[20]

22. Nonidez, J. F.: Studies on the Bones in Avian Rickets: I. Bone Lesions in Chickens Deprived of the Antirachitic Factor After 5 Weeks of Normal Growth, Am. J. Path. **4**:463, 1928.

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24. Wolbach, S. B., and Hegsted, D. M.: Hypervitaminosis A and the Skeleton of Growing Chicks, A. M. A. Arch. Path. **54**:30, 1952.

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Bowing of the bones of the hock joint is the result of loss of strength following disappearance of the bone-lined cartilage tunnels of the metaphyseal region.

Osteogenesis per se is not affected, which explains the thickness of shortened bones resulting from suppressed endochondral bone formation.

Histologic study is required for the pathological characterization of perosis and for the appraisal of experimental results.

The changes in epiphyseal cartilages in manganese and choline deficiencies are almost precisely similar. Minor differences only in amount and character of the matrix and cytology were found by us.

It seems highly probable that both manganese and choline are operative in a common biochemical system in chicks, essential only for epiphyseal cartilage cell metabolism.

The epiphyseal cartilage changes are distinctive, and the name perosis should be restricted to conditions in animals exhibiting the changes we have described.

The histological preparations were made by Mr. John J. Burke; the photomicrographs by Mr. John Carabitses.

BLOOD CHANGES IN MAN FOLLOWING DEATH DUE TO DROWNING

With Comments on Tests for Drowning

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THE FIRST experimental investigation on the mechanism of death in drowning by what might be termed "modern physiological methods" was conducted by Brouardel (1889).[1] He investigated the diagnostic significance of disproportionate intracardiac hemodilution; he concluded that a comparison of the hemoglobin concentration and erythrocyte content of right and left heart blood constituted a practical method for the identification of death caused by drowning. By the two procedures it was observed that the disproportionate dilution of left heart blood occurred after drowning in fresh water. Paltauf (1892) [2] made similar observations.

Carrara (1902) [3] was among the first to apply physical methods to investigate the effects of drowning on the molecular concentration of heart blood. As a result of determination of specific gravity, freezing point, and electrical conductivity of whole blood, he concluded that left ventricular blood is disproportionately diluted after drowning in fresh water and disproportionately concentrated after drowning in sea water.

Revenstorf (1902) [4] proposed cryoscopic examination of blood as of diagnostic value in borderline cases, while Placzek (1903) [5] recommended the diagnostic value of the determination of the specific gravity of blood from the right and left heart.

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2. Paltauf, A.: Einige Bermerkungen über den Tod durch Ertrinken, Berlin. klin. Wchnschr. **29**:298, 1892.

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4. Revenstorf: Über den Wert der Kryoskopie zur Diagnose des Todes durch Ertrinken, München. med. Wchnschr. **2**:1880, 1902.

5. Placzek: Die Blutdichte als Zeichen des Ertrinkungstodes, Vrtljschr. geritchtl. Med. **25**: 13, 1903.

Gettler (1921) [6] presented a test for drowning which depended on the microchemical determination of the chloride concentration of whole blood in the heart chambers. He concluded that in human subjects a difference in the sodium chloride concentration of the two heart chambers exceeding 25 mg. per 100 ml. of blood indicated drowning. In salt water drowning, the left heart chloride exceeds the right by this amount, while the reverse condition occurs in fresh water drowning. It was observed that, the longer the interval between inhalation of water and death, the greater the differences were likely to be.

Since Gettler's observations, the significance of the chloride content of the blood in relation to death by drowning has been the subject of numerous investigations and consequently considerable diversity of opinion regarding the merit of the test. Moritz (1944) [7] mentions that, unless the blood is obtained and analyzed soon after death, differences which might otherwise be of diagnostic value are likely to be masked by postmortem diffusion. He suggested that differences in magnesium concentration may be more reliable than the chlorides in cases of drowning in sea water.

Carrara's measurements of whole-blood specific gravity gave some indication of success as a drowning test. However, he failed to recognize the postmortem settling of blood as a factor to be considered when whole-blood determinations are made. The present authors believe that plasma or serum specific gravities would offer more stable evidence of the disproportionate hemodilution, since the settling effect would be eliminated.

A study of a number of human drowning deaths was undertaken. Comparison was made of the blood from left and right atria, and determinations were made of the following: whole-blood chloride, plasma chloride, hematocrit, plasma specific gravity, hemoglobin, sodium, potassium, and total protein. Similar studies were made of left and right heart blood obtained from persons whose deaths were due to a variety of causes other than drowning.

MATERIALS AND METHODS

Blood samples were obtained from the left and right atria after exposure of the heart by the routine autopsy procedure. The blood was collected from the atria in chemically clean dry 30 cc. syringes through a 13 gauge needle. The blood was then transferred to clean dry 50 ml. glass-stoppered Erlenmeyer flasks. Aliquots were placed in 15 ml. graduated centrifuge tubes and centrifuged at 2,000 rpm for 30 minutes, after which the total volume and packed-cell volume were read. During centrifugation the tubes were capped with nonporous covers to prevent evaporation. The separated plasma layers were drawn off with dry pipettes and used for the determination of the various plasma constituents.

Separate aliquots of the original whole-blood samples were analyzed for chloride concentration using the method of Schales and Schales [8] and checked by the method of Keys.[9] The results are shown in Tables 1 and 2, as well as those of plasma chloride concentrations.

6. Gettler, A. O.: A Method for the Determination of Death by Drowning, J. A. M. A. **77**:1650, 1921.

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TABLE 1.—*Chlorides*

Case No.	Post-mortem Interval, Hr.	Left Atrium				Right Atrium				Left Atrium — Right Atrium		
		Hematocrit, %	Whole Blood, mEq./L.	Plasma, mEq./L.	Reconstituted, mEq./L.	Hematocrit, %	Whole Blood, mEq./L.	Plasma, mEq./L.	Reconstituted, mEq./L.	Whole Blood, mEq./L.	Plasma, mEq./L.	Reconstituted, mEq./L.
Fresh Water Drowning												
1	5	61.4	83.8	107.2	92.0	57.6	85.4	104.3	92.0	— 1.6	+ 2.9	0.0
2	12	74.2	61.1	99.2	78.7	69.2	83.7	97.4	87.7	—21.9	+ 1.8	— 9.0
3	18	....	....	.....	....	....	.....	.....	....	.....	.....	.....
4	18	83.1	80.3	94.4	87.7	61.6	83.1	110.1	92.7	— 2.8	—15.7	— 5.0
5	18	25.6	91.6	98.5	88.8	33.9	82.4	95.1	81.1	+ 9.2	+ 3.4	+ 7.7
6	22	41.5	85.6	100.7	86.2	57.0	94.2	102.2	96.6	— 8.6	— 1.5	—10.4
7	22	98.4	78.7	111.0	97.2	78.0	80.4	119.5	99.5	— 1.7	— 8.5	— 2.3
8	18	54.6	89.6	104.1	98.5	41.0	82.4	89.6	83.3	+ 6.3	+14.5	+10.2
9	21	50.0	79.2	105.8	83.9	30.0	93.6	101.7	91.0	—14.6	+ 1.3	— 7.1
10	18	....	....	.....	....	....	....	.....	....	.....	.....	.....
11	15	....	85.4	.....	....	....	85.5	.....	....	— 0.1	.....	.....
12	5	54.0	83.0	94.6	86.0	55.0	82.9	93.6	85.7	+ 0.1	+ 1.0	+ 0.3
Brackish Water Drowning												
20	18	64.9	91.8	102.0	95.4	44.2	82.8	106.6	85.0	+ 9.0	— 4.6	+10.4
21	12	50.0	97.5	108.0	101.0	34.6	83.8	98.2	81.5	+13.7	+ 9.8	+19.5
22	20	67.2	92.5	106.2	97.8	58.8	81.7	89.7	84.3	+10.8	+16.5	+13.5
23	2	48.5	94.1	103.8	95.1	55.0	83.5	99.2	87.7	+10.6	+ 4.6	+ 7.4
24	48	60.0	97.8	109.6	101.7	43.6	76.6	83.5	77.3	+21.2	+26.1	+24.4
25	?	39.6	101.4	112.1	101.4	51.8	82.2	105.2	87.6	+19.2	+ 6.9	+13.8
26	8	52.4	94.5	107.0	97.8	67.0	87.2	98.5	92.4	+ 7.3	+ 7.5	+ 5.4
27	18	31.1	89.4	97.2	86.3	48.2	82.5	90.0	81.7	+ 6.9	+ 7.2	+ 4.6
28	22	43.5	112.2	.....	....	50.8	81.4	.....	....	+30.8	.....	.....
29	9	71.8	91.6	101.2	95.9	64.4	80.0	98.2	84.8	+11.6	+ 8.0	+11.1
30	14	57.6	88.3	102.9	92.9	70.9	81.3	93.0	86.4	+ 7.0	+ 9.9	+ 6.5
31	17	66.4	89.9	107.1	96.8	71.6	79.5	91.3	84.8	+10.4	+15.8	+12.0
32	18	55.8	91.4	102.5	94.6	50.8	83.3	99.7	86.7	+ 8.1	+ 2.8	+ 7.9
33	27	44.0	103.3	121.3	106.1	50.0	84.5	129.6	93.5	+18.8	— 8.3	+12.6
34	8	62.6	91.5	109.3	98.2	68.0	81.0	101.2	89.0	+10.5	+ 8.1	+ 9.2
35	6	59.3	95.7	.....	....	58.1	83.0	.....	....	+12.7	.....	.....
36	20	23.2	89.9	100.1	82.1	49.6	82.5	90.2	83.9	+ 7.4	+ 9.9	— 1.8
37	19	50.0	95.0	105.0	97.1	40.0	85.0	97.0	85.0	+10.0	+ 9.0	+12.1
38	15	75.2	91.0	107.0	98.4	33.7	90.3	100.5	88.5	+ 0.7	+ 6.5	+ 9.9
39	19	22.0	102.0	118.0	91.7	26.0	93.5	102.5	88.5	+ 8.5	+15.5	+ 3.2
40	15	13.3	103.0	107.0	88.4	31.6	91.5	99.5	89.4	+ 9.5	+ 7.5	— 1.0
41	18	52.4	102.0	104.0	105.0	75.5	83.9	91.9	87.7	+18.1	+12.1	+15.3
42	18	52.0	100.5	.....	....	76.5	79.1	.....	....	+20.6	.....	.....
43	12	34.6	102.3	120.4	101.1	13.6	95.1	101.9	81.9	+ 8.2	+18.5	+19.2
Salt Water Drowning												
90	?	75.0	112.5	.....	....	93.0	77.5	.....	....	+35.0	.....	.....
91	?	80.0	124.1	.....	....	79.0	88.4	.....	....	+35.7	.....	.....
92	4	56.2	79.5	99.9	85.5	52.4	78.5	96.5	82.9	+ 1.0	+ 3.4	+ 2.4

TABLE 2.—*Chlorides of Control Cases*

Case No.	Post-mortem Interval, Hr.	Left Atrium				Right Atrium				Left Atrium — Right Atrium		
		Hematocrit, %	Whole Blood, mEq./L.	Plasma, mEq./L.	Reconstituted, mEq./L.	Hematocrit, %	Whole Blood, mEq./L.	Plasma, mEq./L.	Reconstituted, mEq./L.	Whole Blood, mEq./L.	Plasma, mEq./L.	Reconstituted, mEq./L.
Cardiac Failure												
45	16	82.1	69.6	91.8	80.9	59.6	82.4	90.8	84.8	—12.8	+ 1.0	— 3.9
46	4	79.1	78.7	108.8	93.0	34.8	92.9	105.8	91.7	—14.2	+ 3.0	+ 1.3
47	15	86.0	79.4	111.9	96.8	52.0	83.3	100.1	87.6	— 3.9	+11.8	+ 9.2
Hanging												
50	16	81.4	79.5	92.8	86.4	53.5	82.9	91.4	84.8	— 3.4	+ 1.4	+ 1.6
51	4	63.0	90.6	107.5	90.4	54.6	90.6	103.5	94.2	0.0	+ 4.0	+ 2.2
Electrocution												
60	18	70.8	82.2	106.0	92.7	51.0	89.6	107.0	92.7	— 7.4	— 1.0	0.0
61	4	72.8	83.8	91.2	87.2	57.6	79.0	99.9	85.4	+ 4.8	— 8.7	+ 1.8
Suffocation with Pillow												
70	23	85.6	94.4	106.2	101.8	36.7	82.9	90.0	82.4	+11.5	+18.2	+19.4
Buried in Sand												
75	20	72.4	83.1	93.5	87.7	45.9	81.9	95.4	83.6	+ 1.2	— 1.9	+ 4.1
Ruptured Cerebral Aneurysm												
80	20	40.0	90.5	104.0	90.5	21.0	93.0	103.9	83.1	— 2.5	+ 0.1	+ 7.4

TABLE 3.—*Plasma Analyses* *

Case No.	Specific Gravity			Hemoglobin, Mg./100 Cc.			Proteins, Gm./100 Cc.			K, mEq./L.			Na, mEq./L.			K/Na × 10	
	L.	R.	L. — R.	L.	R.	L. — R.	L.	R.	L. — R.	L.	R.	L. — R.	L.	R.	L. — R.	L.	R.
Fresh Water Drowning																	
1	1.0305	1.0312	—0.0007	400	413	— 13	8.6	9.0	— 0.4	16.5	12.9	+ 3.6	132	132	0	1.3	1.0
2	1.0322	1.0341	—0.0019	...	298	.....	9.5	10.5	— 1.0	....	28.2	.....	...	132	....	...	2.1
3	1.0342	1.0364	—0.0022	...	...	.....	...	....	.....	....	47.8	.....	...	140	....	...	3.4
4	1.0286	1.0432	—0.0146	253	305	— 12	8.1	11.4	— 3.3	28.8	28.6	+ 0.2	87	34	+53	3.3	8.4
5	1.0315	1.0350	—0.0035	83	11	+ 72	8.7	9.9	— 1.2	24.6	28.4	— 3.8	137	131	+ 6	1.8	2.2
6	1.0264	1.0313	—0.0049	138	90	+ 48	7.7	8.5	— 0.8	26.6	31.0	— 4.4	128	122	+ 6	2.1	2.5
7	1.0265	1.0391	—0.0126	900+	183	+ ?	9.1	....	.....	30.9	....	.....	98	...	....	4.1	...
8	1.0284	1.0293	—0.0019	115	106	+ 9	8.1	8.8	— 0.7	24.8	28.5	— 3.7	128	121	+ 7	1.9	2.4
9	1.0320	1.0337	—0.0017	...	...	.....	9.1	9.7	— 0.6	....	....	.....	...	...	....	...	...
10	1.0316	1.0368	—0.0052	...	63	.....	8.5	9.7	— 1.2	....	33.8	.....	...	107	....	...	3.2
11	1.0362	1.0397	—0.0035	...	...	.....	...	....	.....	....	....	.....	...	...	....	...	...
12	1.0236	1.0311	—0.0075	...	...	.....	...	....	.....	9.5	12.9	— 3.4	126	123	+ 3	0.8	1.0
Brackish Water Drowning																	
20	1.0308	1.0328	—0.0020	22	54	+ 32	7.7	8.3	— 2.2	15.0	13.9	+ 1.1	134	144	—10	1.1	1.0
21	1.0229	1.0289	—0.0060	...	5	.....	...	....	.....	....	8.5	.....	...	138	....	...	0.6
22	1.0286	1.0357	—0.0071	10	10	0	7.6	9.0	— 1.4	13.0	14.7	— 1.7	135	134	+ 1	1.0	1.1
23	1.0273	1.0291	—0.0018	121	54	+ 67	6.9	8.2	— 1.3	12.0	13.0	— 1.0	147	140	+ 7	0.8	0.9
24	1.0234	1.0347	—0.0113	...	...	.....	...	....	.....	....	....	.....	...	...	....	...	...
25	1.0210	1.0283	—0.0073	30	86	— 56	5.7	7.9	— 2.2	6.4	9.5	— 3.1	128	133	— 5	0.5	0.7
26	1.0301	1.0319	—0.0018	...	...	.....	...	....	.....	....	....	.....	...	...	....	...	...
27	1.0358	1.0428	—0.0070	...	...	.....	...	....	.....	....	....	.....	...	...	....	...	...
28	1.0222	1.0380	—0.0158	...	...	.....	...	....	.....	22.1	23.4	— 1.3	124	118	+ 6	1.8	2.0
29	1.0246	1.0308	—0.0062	15	19	— 4	7.4	9.6	— 2.2	17.2	27.1	— 9.9	125	127	+ 2	1.4	2.1
30	1.0312	1.0323	—0.0011	113	125	— 12	8.8	9.3	— 0.5	23.0	28.3	— 5.3	56	163	—107	4.1	1.7
31	1.0291	1.0348	—0.0057	226	148	+ 78	8.1	11.4	— 3.3	24.0	37.0	—13.0	118	112	+ 6	2.0	3.3
32	1.0302	1.0345	—0.0043	83	15	+ 68	7.9	8.7	— 0.8	29.2	29.3	— 0.1	144	132	+12	2.0	2.2
33	1.0278	1.0384	—0.0106	234	148	+ 86	7.7	11.8	— 4.1	18.4	20.6	— 2.2	128	130	+ 2	1.4	1.6
34	1.0221	1.0362	—0.0141	76	210	—134	6.8	11.0	— 4.2	10.9	31.2	—20.3	92	127	—35	1.2	2.5
35	1.0203	1.0311	—0.0108	...	...	.....	...	....	.....	14.7	18.5	— 3.8	141	149	— 8	1.0	1.2
36	1.0317	1.0349	—0.0032	...	...	.....	...	....	.....	18.9	21.8	— 2.9	136	121	+15	1.4	1.8
37	1.0277	1.0307	—0.0030	...	...	.....	...	....	.....	19.5	20.2	— 0.7	142	140	+ 2	1.4	1.4
38	1.0246	1.0325	—0.0079	...	...	.....	...	....	.....	14.6	21.5	— 6.9	119	139	—20	1.2	1.5
39	1.0251	1.0293	—0.0042	...	...	.....	...	....	.....	12.2	15.9	— 3.7	140	143	— 3	0.9	1.1
40	1.0285	1.0298	—0.0013	...	...	.....	...	....	.....	11.8	18.3	— 6.5	139	134	+ 5	0.8	1.4
41	1.0263	1.0363	—0.0100	...	...	.....	...	....	.....	....	....	.....	...	...	....	...	...
42	1.0372	1.0377	—0.0005	...	...	.....	...	....	.....	....	....	.....	...	...	....	...	...
43	1.0273	1.0338	—0.0065	...	...	.....	...	....	.....	....	....	.....	...	...	....	...	...
Salt Water Drowning																	
90	1.0319	1.0468	—0.0149	...	...	.....	...	....	.....	....	....	.....	...	...	....	...	...
91	1.0275	1.0438	—0.0163	...	...	.....	...	....	.....	....	....	.....	...	...	....	...	...
92	1.0289	1.0328	—0.0039	...	...	.....	...	....	.....	....	....	.....	...	...	....	...	...

* L., left atrium; R., right atrium.

TABLE 4.—*Plasma Analyses of Control Cases* *

Case No.	Specific Gravity			Hemoglobin, Mg./100 Cc.			Proteins, Gm./100 Cc.			K, mEq./L.			Na, mEq./L.			K/Na × 10	
	L.	R.	L. — R.	L.	R.	L. — R.	L.	R.	L. — R.	L.	R.	L. — R.	L.	R.	L. — R.	L.	R.
Cardiac Failure																	
45	1.0373	1.0346	+0.0026	...	13	.....	12.1	10.2	+ 1.9	31.4	18.6	+12.8	142	142	0.0	2.2	1.3
46	1.0315	1.0302	+0.0013	355	16	+339	9.8	8.2	+ 0.6	8.9	11.3	— 2.4	138	138	0.0	0.6	0.8
47	1.0322	1.0308	+0.0014	48	5	+ 43	8.8	8.1	+ 0.7	15.9	13.9	+ 2.0	142	137	+ 5.0	1.1	1.0
Hanging																	
50	1.0374	1.0331	+0.0043	...	...	.....	...	....	.....	18.2	17.8	+ 0.4	111	130	−19.0	1.6	1.4
51	1.0313	1.0306	+0.0007	23	24	— 1	8.0	7.3	+ 0.7	14.7	21.0	— 6.3	157	145	+12.0	0.9	1.4
Electrocution																	
60	1.0377	1.0357	+0.0020	54	23	+ 31	9.6	8.2	+ 1.4	33.0	30.1	+ 2.9	115	112	+ 3.0	2.9	2.7
61	1.0337	1.0308	+0.0029	29	30	— 10	10.0	8.8	+ 1.2	20.2	18.2	+ 2.0	177	175	+ 2.0	1.1	1.0
Suffocation with Pillow																	
70	1.0365	1.0363	+0.0002	168	103	+ 65	...	10.8	.....	....	41.6	.....	...	121	....	...	3.4
Buried in Sand																	
75	1.0330	1.0326	+0.0003	77	25	+ 52	9.3	9.1	+ 0.2	28.1	26.7	+ 1.4	152	150	+ 2.0	1.8	1.8
Ruptured Cerebral Aneurysm																	
80	1.0300	1.0290	+0.0010	...	...	.....	...	....	.....	13.4	11.7	+ 1.7	139	136	+ 3.0	1.0	0.9

* L., left atrium; R., right atrium.

The specific gravities of the left and right heart plasma were determined by the method of Hamilton and Barbour [10] and checked by the copper sulfate method.[11] Plasma proteins were determined (micro-Kjeldahl method) and checked with values calculated from specific gravity determinations. Sodium and potassium concentrations were obtained with a Beckman flame photometer. Hemoglobin was determined photometrically by the benzidine reaction.[12] The results of these determinations are recorded in Tables 3 and 4.

COMMENT

The results given in Tables 1, 2, 3, and 4 show that the differences in the chloride concentration of the whole blood taken from the left and right heart chambers do, according to Gettler's criteria, demonstrate the cause of death in all but one of the brackish water drownings and in two of the three salt water drownings. However, five of the fresh water drownings would have been inaccurately diagnosed. Furthermore, of the 10 control cases, 3 would have been diagnosed as fresh water drownings and two as salt or brackish water drownings.

It was thought that these discrepancies in diagnosis may have been the result of partial settling of blood cells in the heart during the postmortem interval, with

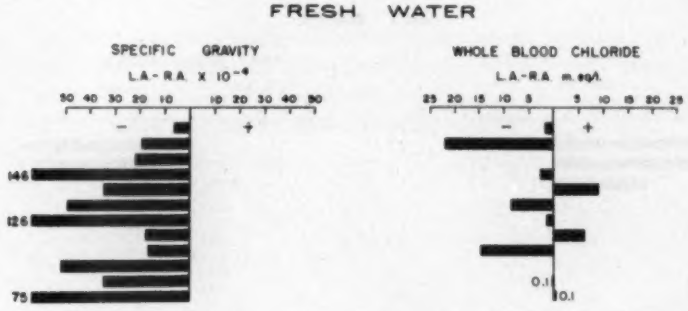

Chart 1.—Plasma specific gravity differences and whole-blood chloride differences observed in cases of drowning in fresh water.

such settling proceeding differently in the two sides. This would lead to artifact, in that the blood samples obtained would not be truly representative of whole blood. An attempt was made to correct this by determining the hematocrit and plasma chloride concentration, as well as the whole-blood chlorides. From these data the chloride concentration of reconstituted blood (on the basis of a 40% hematocrit value) was calculated. The differences between the reconstituted values of the left and right heart blood show even more erratic behavior than the whole-blood values originally determined. Thus it may be stated that the whole-blood and plasma chloride behave in an unpredictable fashion in both drownings and controls and that no correlation exists between differences in the left and right heart chloride concentrations and the clinical history.

10. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity, J. Biol. Chem. **69**:625, 1926.

11. Phillius, R. A.; Van Slyke, D. D.; Hamilton, P. B.; Dole, V. P.; Emerson, K. Jr., and Archibald, R. M.: Measurement of Specific Gravities of Whole Blood and Plasma by Standard Copper Sulfate Solutions, J. Biol. Chem. **183**:305, 1950.

12. Bing, F. C., and Baker, R. W.: The Determination of Hemoglobin in Minute Amounts of Blood by Wu's Method, J. Biol. Chem. **92**:589, 1931.

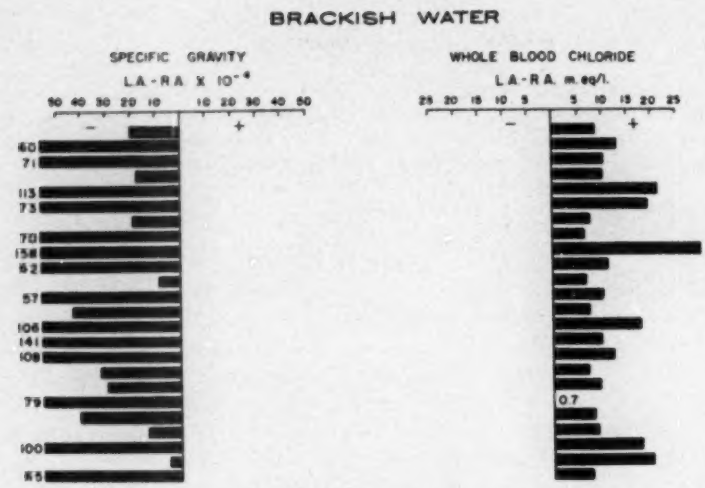

Chart 2.—Plasma specific gravity differences in whole-blood chloride differences observed in cases of drowning in brackish water.

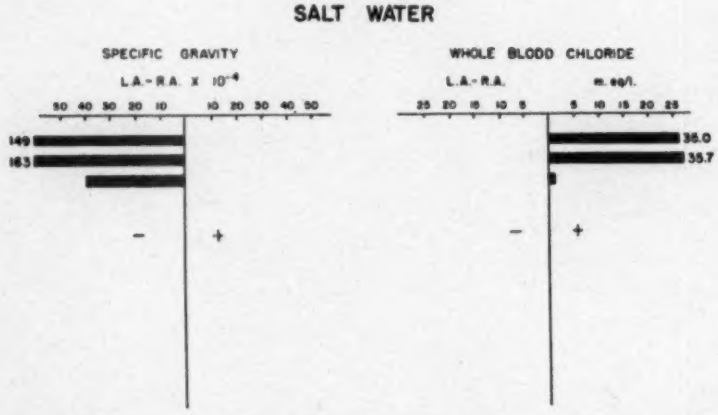

Chart 3.—Plasma specific gravity differences and whole-blood chloride differences observed in cases of drowning in salt water.

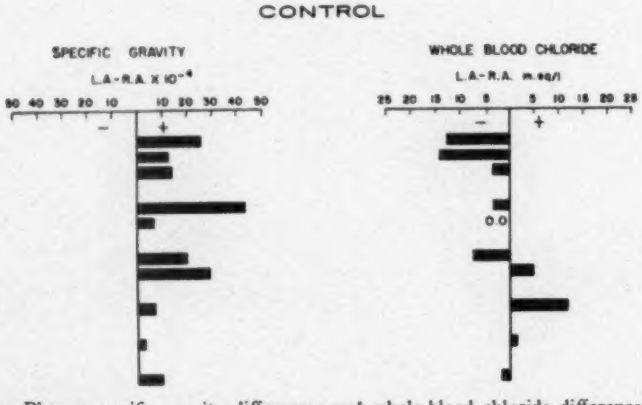

Chart 4.—Plasma specific gravity differences and whole-blood chloride differences observed in control cases.

Considerable overlapping of values, with no significant differences between groups, is shown in the ratios of plasma potassium to sodium, as well as in the individual sodium and potassium values. This is also true of plasma hemoglobin. Hence, neither of these factors can be used for diagnostic purposes.

The plasma specific gravities recorded in Tables 3 and 4 show lower values in the left atrium than in the right atrium following drowning in fresh, brackish, and salt water. In instances of death not due to drowning (Table 2), the specific gravity of the left atrial plasma is greater than that of the right. Additional control cases are being studied to determine whether this same condition prevails in all other types of sudden death. Comparison of the plasma specific gravity differences and the whole-blood chloride differences observed in cases of drowning and in control cases is graphically presented in Charts 1, 2, 3, and 4.

CONCLUSIONS

1. Diagnosis of death due to drowning based on the difference between whole-blood or plasma chloride concentrations in the left and right heart is less reliable than the determination of plasma specific gravity difference in the two sides of the heart.

2. In all cases of drowning, irrespective of the salinity of the water, the specific gravity of the left atrial plasma is less than that of the right atrial plasma.

3. In the nondrowning control deaths studied thus far, the specific gravity of the left atrial plasma was higher than that of the right atrial plasma.

Russell S. Fisher, M.D., William V. Lovitt, M.D., Stephen M. Cain, and John U. Bures gave their assistance in this study.

CANCER ASSOCIATED WITH OVARIAN STROMAL HYPERPLASIA

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SINCE relationships between both cancers of the endometrium [1] and cancers of the breast [2] and abnormal ovaries have been reported in the literature, a study has been constructed to determine the condition of ovaries in women dying with cancers at other sites. The pathologic ovarian change most commonly observed previously has been the presence of nodular hyperplastic masses of ovarian cortical stroma, either premenopausal or postmenopausal, in slightly over 80% of women with either of these two types of cancer. Steroid formation of functional importance has been ascribed to the hyperplastic stromal masses. G. V. Smith, who discovered this lesion, has recently pointed out that it has not been proved that the hyperplastic stroma is or is not producing estrogens.[3] Some evidence would favor progesterone synthesis.

Microscopically the diagnosis of hyperplastic ovarian cortical stroma is based upon the presence of abnormally nodular whorled cellular stromal masses which thicken the cortex and often extend into the medullary collagenous tissue. The severer degrees of cortical stromal hyperplasia are associated with nodules from at least 3 to 6 mm. in diameter. The less marked instances are characterized by nodules of cortical stroma 1 to 3 mm. in diameter. Sometimes the postmenopausal ovary retains its cortical stroma without undergoing either atrophy or nodular hyperplasia. The smooth superficial sheet of cortical stroma in this situation measures from 0.5 to 1.5 mm. in thickness. No ovary which lacked a definitely nodular type of stromal overgrowth was considered to have cortical stromal hyperplasia. Thecomatosis and cortical granulomas usually accompany the more extreme hyperplasias.

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From the Laboratories of Pathology, Cancer Research Institute, Harvard Cancer Commission, New England Deaconess Hospital, Boston, and Pondville Hospital (Massachusetts Department of Public Health), Walpole, Mass.

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A prior study suggested that some other cancers in women might be significantly often associated with ovarian cortical stromal hyperplasia. A group of 600 autopsies of women from the files of the New England Deaconess Hospital was reviewed to examine the prepared ovarian sections. Following histologic examination, the chief disease conditions were correlated with the presence of ovarian abnormalities. Particular interest was directed to women dying with cancer.

In a few types of cancer an increased frequency of ovarian stromal hyperplasia was suggested. Attempt was made in these instances to collect all available data on cases in which autopsies were performed, with ovarian sections from the same hospital files, and to augment these with data on cases from Pondville Hospital (Massachusetts Department of Public Health). The resulting material was analyzed statistically. In addition, two types of cancer without any apparent unusual frequency of associated ovarian stromal hyperplasia were similarly investigated, using all available cases.

The groups of cancer cases subjected to final analysis were all pathologically proved by review of histologic material available. The average ages of the cancer patients at death in the major groups were from 52 to 59 years. From considerations of uniformity of sex, age, and pathologic type of cancer, the groups were rather homogeneous. The non-neoplastic control material differed as little as possible in age incidence. So far as the present knowledge of the development of ovarian cortical stromal hyperplasia goes, no complicating extrinsic factors are involved in the development of this ovarian condition.

RESULTS

A significantly higher proportion of ovaries had cortical stromal hyperplasia accompanying cancers of the esophagus, stomach, and lung as compared to the control series (Table).

In contrast, cancers of cervix and colon had about the same rates as occurred in a series of noncancer controls. In the previous investigation the frequency in controls was 37.6% with cortical stromal hyperplasia.

No difference was observed between the material from the two hospitals for any of the selected cancer sites. It was assumed, therefore, that within each site the data for Pondville and New England Deaconess Hospitals could be combined. When significance tests were applied to these combined data, it was found that the frequency of occurrence of cortical stromal hyperplasia in patients with carcinomas of the stomach, lung, and esophagus was significantly higher than in patients with carcinomas of the cervix and colon, while within the high-rate group and within the lower rate group no significant differences were observed. Thus it would appear that the cases with cortical stromal hyperplasia among these selected sites of primary carcinomas were divisible into two groups, one with an overall rate of 69.3% (standard error $\pm 4.9\%$) and the other with an overall rate of 34.0% (standard error $\pm 3.3\%$), and that the statistical difference between these two levels was extremely significant.

Groups of patients dying with cancers of the gall bladder, urinary bladder, kidney, nasopharynx, nasal sinus, or salivary gland and with malignant gliomas were also reviewed. No group was large enough to warrant a statistical conclusion. Renal cell carcinoma was accompanied by ovarian cortical stromal hyperplasia in seven of eight cases collected.

Scrutiny of the severity of cortical stromal hyperplasia was undertaken, with particular reference to increased lipoid content, so-called thecomatosis, or hyperthecosis. Cases were divided into more marked and less marked on this basis.

Carcinoma of the stomach had 14 with more pronounced changes of 32 assayed (44%); lung carcinoma, 3 of 15 (20%); carcinoma of the esophagus, 6 of 10 (60%); carcinoma of the cervix, 14 of 30 (47%), and carcinoma of the colon, 12 of 38 (32%).

COMMENT

The importance of hormonal stimuli in the production of cancers of endometrium and breast is generally recognized. Addition of three other types of carcinoma in women, associated frequently with ovarian cortical stromal hyperplasia, suggests

Per Cent of Autopsies Showing Cortical Stromal Hyperplasia and Standard Errors of Percentages

Primary Site	Hospital *	No. of Cases	Cortical Stromal Hyperplasia, %	Standard Error, %
Stomach	N. E. D. H.	33	63.6	± 8.4
	Pondville	18	83.3	± 8.8
	Combined	51	70.6	± 6.4
Lung	N. E. D. H.	18	61.1	±11.5
	Pondville	5	80.0	±17.9
	Combined	23	65.2	± 9.9
Esophagus	N. E. D. H.	5	80.0	±17.9
	Pondville	9	66.7	±15.7
	Combined	14	71.4	±12.1
Cervix	N. E. D. H.	31	32.3	± 8.4
	Pondville	66	30.3	± 5.7
	Combined	97	30.9	± 4.7
Colon	N. E. D. H.	76	31.6	± 5.3
	Pondville	33	48.5	± 8.7
	Combined	109	36.7	± 4.6
Stomach-Lung-Esophagus		88	69.3	± 4.9
Cervix-Colon		206	34.0	± 3.3
Non-neoplastic controls		133	37.6	± 4.2

* Material from New England Deaconess Hospital (1930-1951) and Pondville Hospital (1928-1952).

a consideration of the endocrine factors in their development also. The incidences of carcinomas of lung, esophagus, and stomach in men are three to six times those in females.[4] The frequency of pathologically demonstrable endocrine gland lesions accompanying the fewer cancers of this type observed in women affords interesting material for speculation.

Recent cytologic studies[5] have correlated ovarian stromal hyperplasia with the presence of increased numbers of hypertrophic amphophile cells in the anterior pituitary lobe. This adds further evidence of pituitary participation in the endocrine imbalances associated with, and possibly predisposing to, these neoplasms.

The increase in pituitary hypertrophic amphophile cells was first observed by Mellgren in Cushing's adrenogenital syndrome.[6] The adrenocortical dysfunction felt

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at present to provide the basis of Cushing's syndrome thus also casts a shadow of etiologic suspicion over cancers of endometrium, breast, stomach, esophagus, and lung in women. No statistical evidence has implicated this syndrome in carcinomas of cervix or colon.

The lack of uniform occurrence with various cancers suggests that ovarian stromal hyperplasia is not merely secondary to an established carcinoma and that it does not result from nonspecific pituitary stimulation secondary to tissue necrosis at the cancer site or elsewhere.

Histologic examination of the adrenal glands has failed to demonstrate abnormalities beyond the nodular cortical architecture of long disputed significance. The lack of abundant pituitary material has prevented our extensive study of its histology, but cell counts are planned on those pituitary glands available as part of another project.

No doubt some other cancers may be associated statistically with an abnormally increased frequency of ovarian cortical stromal hyperplasia. The chance selection of types of cancer available in our material in sufficient numbers for analysis would preclude a generalization concerning other neoplasms.

SUMMARY

Statistical investigation of the cancers found to be associated commonly with ovarian cortical stromal hyperplasia has revealed significantly higher frequencies accompanying carcinomas of the lung, esophagus, and stomach in women as compared to carcinomas of cervix or colon and to non-neoplastic control cases. A majority of breast and endometrial cancers had previously been shown to share this abnormality. The accompanying pituitary changes and inferred adrenocortical dysfunction may be of carcinogenic importance.

IS HISTOLOGIC GRADING OF COLON CARCINOMA A VALID PROCEDURE?

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VARIATION in the growth potential, invasive qualities, and metastatic capacities of neoplastic diseases has occasioned intensive study, with the accumulation of a vast body of data. The analysis of this information for prognostic implications has occupied the attention of almost every individual concerned with the clinical study of tumors.

It has been widely conceded that, with some exceptions, tumors which exhibit failure of cellular maturation are generally of aggressive nature and those with cytologic maturity are more readily controlled by therapeutic measures. Utilizing reproducible histologic criteria, these qualities have been formalized into arbitrary classes or grades of differentiation. The application of statistical methods to follow-up data has indicated that prognostic significance may indeed be attributed to these classifications. Among the early investigators to use this method, Broders [1] devised a system of grading of squamous-cell carcinoma which in due course became widely adopted. Extending its application to neoplasms in general, its originator [2] and others,[3] soon made use of the method in carcinoma of the colon.

Much evidence has appeared in support of the validity of microscopic grading of carcinoma as a means of not only estimating the outcome but also determining the therapeutic approach.[4] It has become apparent, however, that for individual patients

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with any given grade of carcinoma accurate predictability was impossible. Indeed, if one contrasts the survival rates of several independent analysts, it becomes quickly obvious that, though group trends parallel each other, percentages of survival in each group do not. By some this was interpreted to be a result of varied duration; to others the variable appeared to be the extent of intramural invasion,[5] and to still others it appeared to be correlated with the presence or absence of lymph node metastasis.[6] Moreover, there were some who were less prone to accept uniformity as a characteristic of neoplastic growth. These individuals [7] raised the salient question of whether the histologic structure in an area casually selected for microscopic study was truly representative of the remainder of the lesion. Willis [8] considers this a valid objection to the practice of grading carcinoma and states, "In view of the great variety of cellular structure to be seen in different members of most classes of tumours, and often in a single tumour, the application of Broders' method seems to me to be largely guesswork," and also ". . . attempts at precise numerical grading . . . for prognostic purposes are wasted effort." However, Warren [9] and others, although recognizing a modicum of variation, have felt that tumor patterns in individual lesions have been remarkably constant. Recently, Broders [10] has written, "I should like to state that as a rule there is practically the same grade of malignancy throughout a carcinoma."

Despite this controversy the numerical grading of tumors continues to have wide usage. Some have utilized it merely as an academic exercise, but the belief continues among many that the method is a valid and useful one.

Our interest in this subject was aroused in the course of a broad investigation of the early manifestations of carcinoma in its major visceral sites. Evidence was adduced in relation to carcinoma of the stomach [11] and of the lung [12] that not only were tumors in these regions often the products of multiple foci of origin but also (and perhaps as a direct result of this mode of development) they were exceedingly pleomorphic in their cytologic patterns. It was clearly demonstrated that wide variation existed in the histologic structure of different portions of the same tumor. It seemed to be of some purpose to apply a similar approach to carcinoma of the colon.

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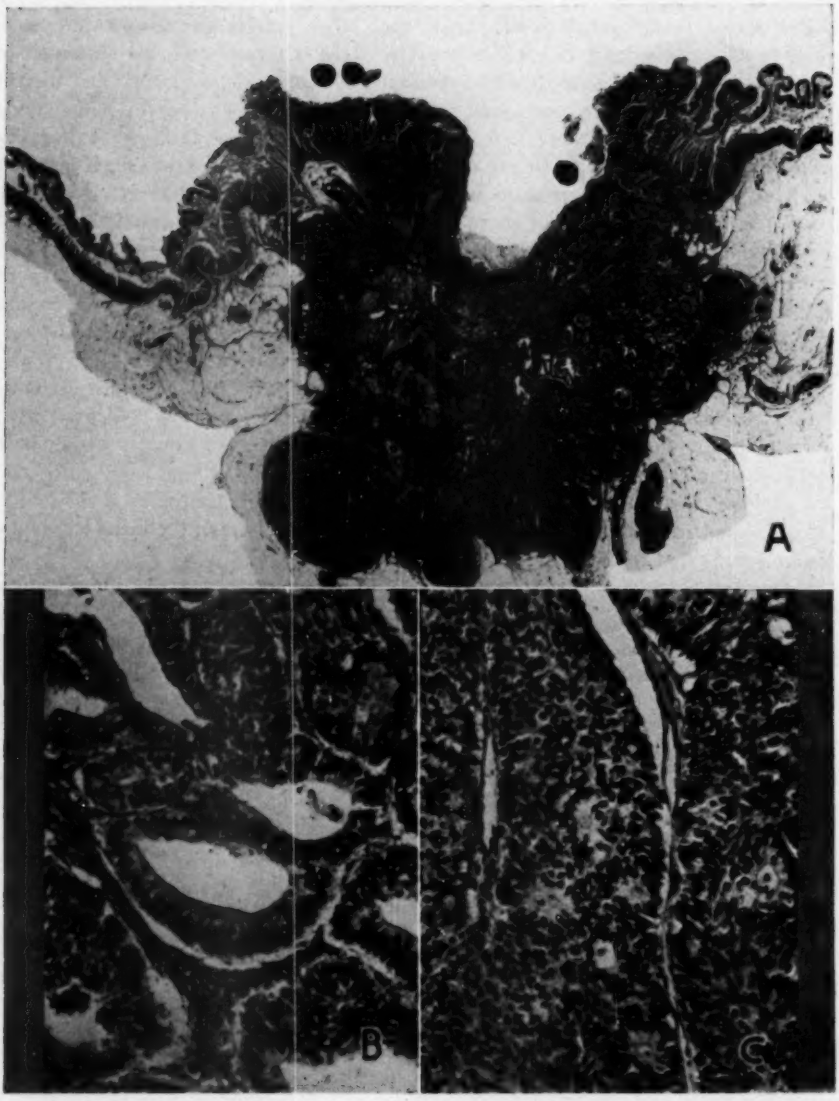

Fig. 1.—*A*, a large tissue section of an ulcerated infiltrating colon carcinoma. The dots indicate areas of varied structure; × 1.3. *B*, an area designated by one of the dots showing a well-differentiated adenocarcinoma; × 160. *C*, another area in the same tumor exhibiting adenocarcinoma with poor differentiation; × 160.

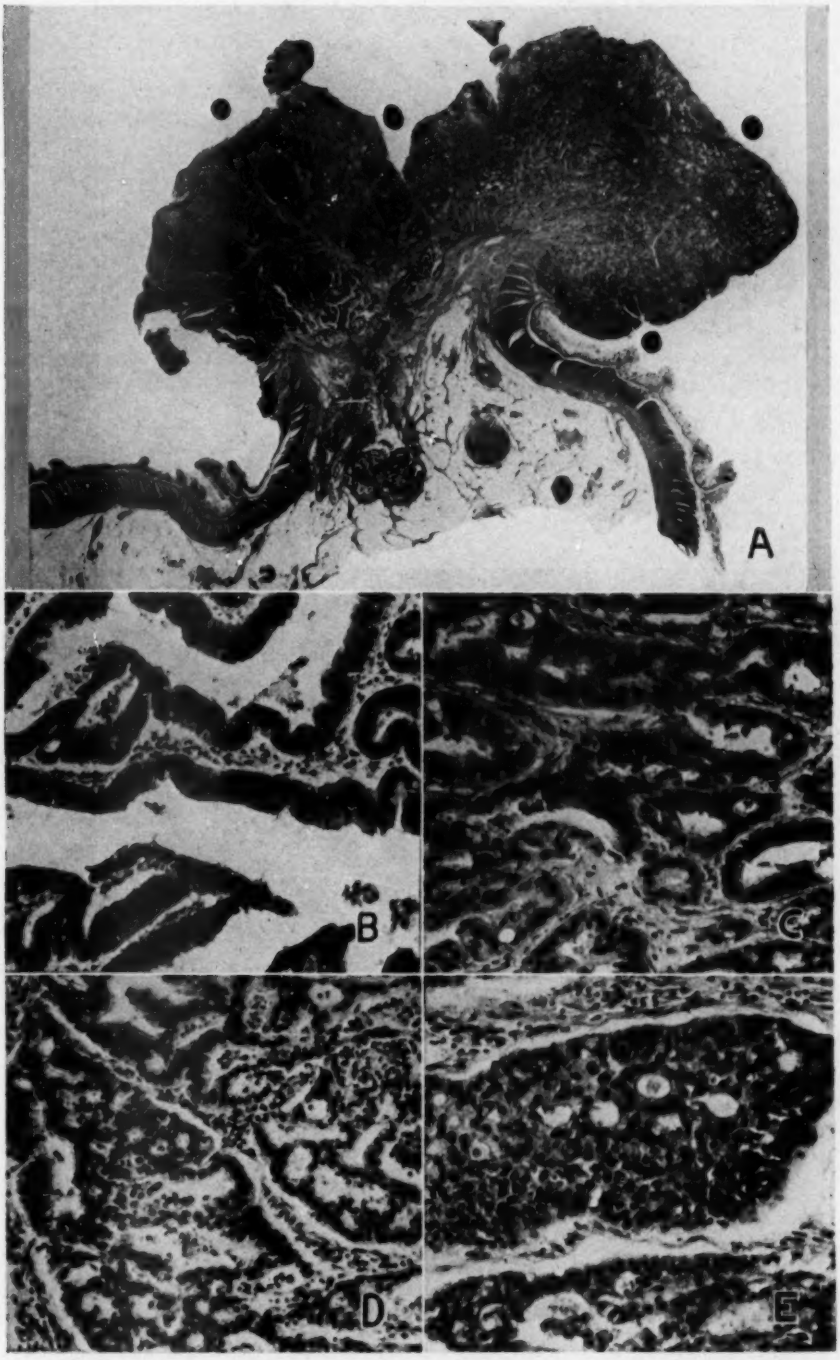

Fig. 2.—*A*, a bulky polypoid colonic carcinoma. Dots indicate four areas illustrated at higher magnification; × 1.3. *B*, well-differentiated adenocarcinoma; × 160. *C*, moderately well-differentiated adenocarcinoma with a scirrhous component; × 160. *D*, cellular adenocarcinoma with abundant secondary acinus formation; × 160. *E*, undifferentiated adenocarcinoma with infrequent acinus formation; × 160.

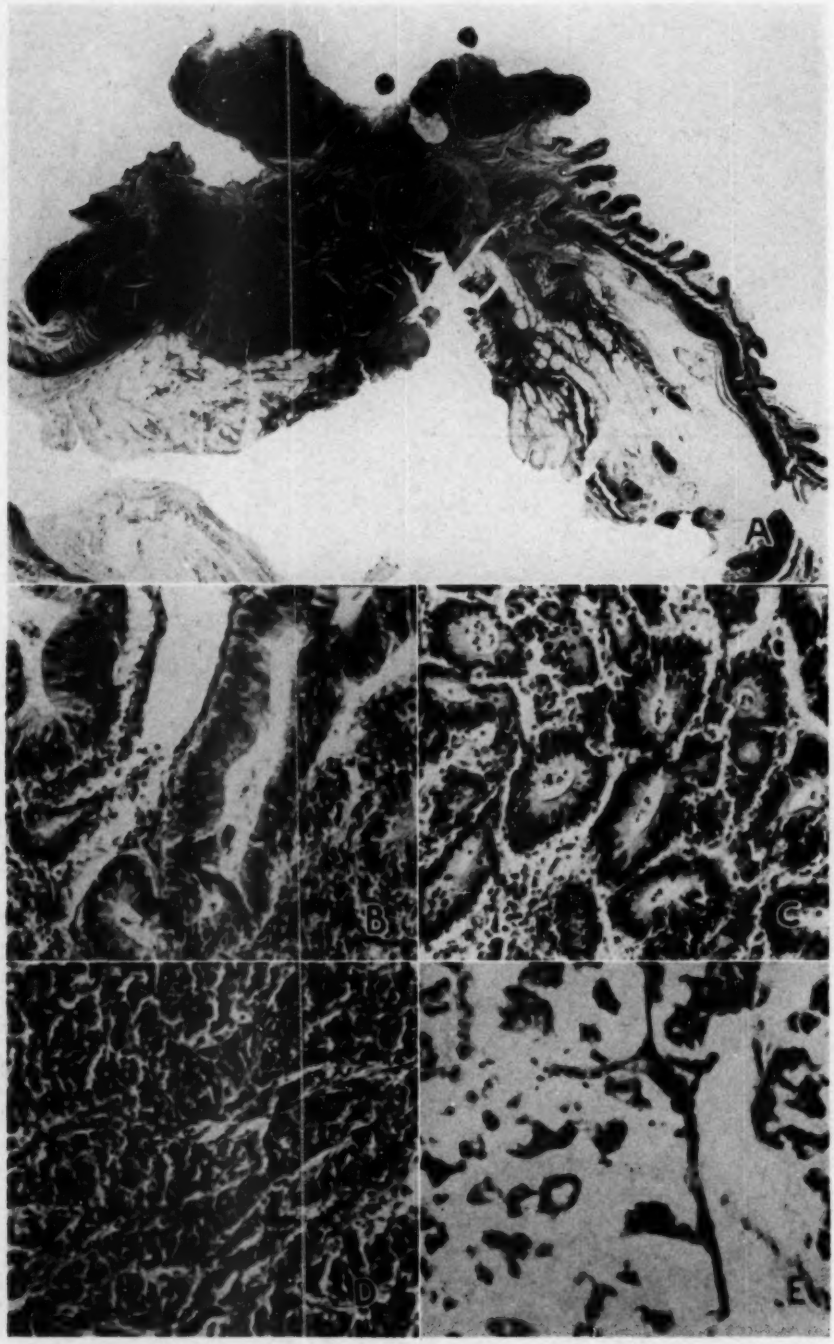

Fig. 3.—*A*, a sessile polypoid carcinoma with wide infiltration. Some indication of the varied histologic pattern is discernible without magnification; × 1.3. *B*, well-differentiated acinus formation. This area is at a juncture with undifferentiated small-cell carcinoma, some of the components of which may be seen in the lower portion of the photomicrograph; × 160. *C*, a focus composed of small but moderately well-differentiated acini; × 160. *D*, wholly undifferentiated small-cell carcinoma without organoid pattern; × 160. *E*, colloid carcinoma. This area may be seen in the low-power photomicrograph (Fig. 3 *A*) as a nonstained focus at the juncture of right and central third of the tumor; × 160.

MATERIALS AND METHOD

The material investigated consisted of 30 examples of adenocarcinoma of the colon and rectum. All were surgically resected specimens. They exhibited variable range in breadth and bulk.

Each specimen was opened promptly and circumspectly with a view to preserving the neoplasm intact. It was then photographed, and a small segment was removed for routine diagnostic purposes. The remainder of the specimen, pinned flat on a cardboard, was fixed by immersion in 10% formalin. Two or three parallel blocks of tissue were prepared through the entire length of the carcinoma and the adjacent mucosa. The blocks were 10 to 12 cm. long. Embedded in paraffin, the sections were cut on a sliding microtome at a thickness of 5 to 8 μ. Staining was carried out with hematoxylin and eosin.

The large tissue sections were first carefully surveyed with a dissecting microscope, and areas of structural variation were marked for further study. A similar survey was then carried out under higher magnification, with special attention being directed to the areas previously marked.

OBSERVATIONS

Although it had been anticipated that some variation of structural pattern would be encountered, the pleomorphism actually observed was quite unexpected. As shown in the Table, only eight of the tumors exhibited a consistent arrangement throughout.

Variation of Histologic Structure

No. of Patterns	No. of Tumors
1	8
2	5
3	6
4	7
5	2
6	2

These, in general, revealed a relatively well-differentiated process classifiable by Broders' criteria as Grade II. Among the remaining 22 carcinomas, however, the cytologic and structural variation of the tumor from one portion to another was often striking. The range was from two to six types of lesions, and in several there was more than one focus of identical pattern separated by segments of dissimilar structure. As in carcinoma of the stomach and lung, the lines of juncture of different types of structure often revealed some intermixture but as a rule were relatively sharp. The variation was not solely a matter of morphologic arrangement, for the degrees of differentiation, hence the grade, varied with equal frequency (Fig. 1). Applying the cytologic criteria commonly utilized for this purpose, it was possible to detect the full gamut of degrees of differentiation in a single lesion (Fig. 2). Several of the tumors, in addition, exhibited colloid carcinoma as well (Fig. 3).

COMMENT

These data clearly indicate that a random section of colonic carcinoma cannot be considered necessarily representative of the remainder of the tumor. Little more than one quarter of the lesions in this small but significant series has revealed a single consistent histologic pattern throughout. It naturally follows that the usual specimen for biopsy or the sampling carried out as a routine in the practice of surgical pathology may not be considered adequate for prognostic purposes. The evidence

of this study does not support the contention that the microscopic structure of carcinoma of the colon is particularly uniform in a given tumor. The contrary is obviously the case.

Under the circumstances it is difficult to weigh the data arising from the premise of structural homogeneity in colon carcinoma. Perhaps a critical reevaluation of one or more coincidental factors may serve to resolve the inconsistency which exists. Such factors may be the pretreatment duration of disease, anatomic location, degree of intramural encroachment, relationship to vascular or lymphatic channels, proximity to serous surfaces, extent of lymph node metastasis, or adequacy and character of therapeutic measures.

SUMMARY

Thirty surgically resected carcinomas of the colon have provided material for the microscopic study of large tissue sections encompassing the entire length of the tumors. A consistent histologic structure was found in only eight specimens (27.7%). The remainder exhibited a range of two to six different patterns, the majority of which revealed varying degrees of differentiation. Since almost three-fourhs of this series of tumors exhibited such cytologic variation, the utilization of microscopic grading as a means of determining prognosis seems to be without basis.

EFFECT OF STREPTOCOCCAL GROWTH PRODUCTS ON DEVELOPMENT OF EXPERIMENTAL VIRAL ARTHRITIS

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THE EXPERIMENTS to be described in this paper were undertaken for two reasons: 1. The inflammatory character of rheumatic and rheumatoid arthritis has aroused speculation that they may have an infectious etiology. Although no virus has been isolated in either condition, Gordon [1] concluded only a few years ago that "the chief infective agent in rheumatic fever is almost certainly a virus." Any theory of the pathogenesis of rheumatic fever must account for the role of the hemolytic Streptococcus. McCarty [2] has declared recently that the major basis for the ever-recurring thesis that rheumatic fever is caused by a virus-like agent, perhaps specifically provoked by a preceding streptococcal infection, is the fact that rheumatic activity is perpetuated after the infection with the organism has subsided. It is his belief that there is little convincing evidence that such a virus exists.

Because of certain similarities of experimental viral carditis to the lesions seen in hearts affected by rheumatic fever, Pearce [3] investigated the effect of streptolysin O in causing localization of systemic viruses in the hearts of rabbits. The administration of this toxin increased the incidence and severity of the cardiac lesions. Streptolysin O is only one of several substances that potentiates the development of viral carditis in rabbits. If it could be demonstrated that certain growth products of the hemolytic Streptococcus can cause viruses to localize within joints, a hypothetical mechanism for the development of rheumatic arthritis might be provided. That such a phenomenon might eventuate was suggested by the experiments of Gordon,[1b] confirmed by Dyson,[4] in which intravenous inoculation of hemolytic Streptococci with

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vaccinia virus in rabbits resulted in the development of vaccinial periarthritis. There is some evidence that streptococcal,[5] as well as testicular,[6] hyaluronidase enhances the development of dermal vaccinia.

2. There is virtually no information concerning the pathology of arthritis occurring during the course of other known diseases of viral etiology. So far as we are aware, viral arthritis has not been the subject of previous experimental consideration.

METHODS AND MATERIALS

The following two types of experiments were performed: (1) induction of arthritis by direct inoculation of viruses into the knees of rabbits, (2) attempts to modify the synovial membrane so that systemic viral infections might come to involve the joints. Young New Zealand male rabbits weighing 2,000 to 2,500 gm. were employed. The experiments were carried out under light anesthesia with pentobarbital (Nembutal) and ether. Intra-articular instillations were

TABLE 1.—*Lesions Produced by Inoculation of Viruses into Joints of Rabbit* *

Experiment No.	Procedure: Right Knee, Virus, Ce.	Procedure: Left Knee, Virus, Ce.	Procedure: Extra-articular Virus	Day Killed	Postmortem Findings: Heart	Postmortem Findings: Right Knee	Postmortem Findings: Left Knee
1	Vaccinia s, 0.1 to 0.2	—	—	3	++ m	+++ sy and p	—
2	Vaccinia s, 0.3	—	—	4	+++ m	++++ sy and p	—
3	Vaccinia s, 0.5	—	—	4	+++ m	++++ sy and p	—
4	Vaccinia f, 0.2	Vaccinia f, 0.1	—	5	+ m	++ sy and p	++ sy and p
5	Vaccinia s, a, 0.5	Vaccinia s, a, 0.2	—	4	- -	± nonspecific sy	++ nonspecific sy
6	—	—	Vaccinia s, intratesticular	4	—	—	Normal
7	Myxoma s, 0.2	—	—	8	—	++++ sy and p	+ nonspecific p
8	—	Myxoma s, 0.5	—	7	—	+ p	++++ sy and p
9	Myxoma f, 0.5	—	—	8	—	++++ sy and p	+ to ++ nonspecific sy
10	Myxoma s, a, 0.5	Myxoma s, a, 0.4	—	7	—	± nonspecific sy	± nonspecific sy
11	Virus III s, 0.5	Virus III f, 0.5	—	2	+ m	++ sy	± nonspecific sy
12	Virus III s, 0.5	Virus III f, 0.5	—	3	—	+ sy and p	± nonspecific sy
13	Virus III s, 0.5	Virus III f, 0.4	—	4	—	++ sy	+ nonspecific sy

* Key to symbols: s, suspension; f, filtrate; a, autoclaved; m, myocarditis; sy, synovitis; p, periarthritis.

made with a 25 gauge needle. The skin over the joint was prepared by application of tincture of iodine followed by 70% alcohol. Precautions against bacterial infection were taken. When the animals were killed, the joints were incised as aseptically as possible; a swab stick was touched to the synovial membrane, and culture was made on Brewer's thioglycollate medium. In no instance was bacterial contamination demonstrated.

Three viruses were made available by Dr. John M. Pearce. These were a highly virulent strain of lapine vaccinia virus, infectious myxoma virus, and virus III. The viruses were inoculated in the form of a crude suspension of infected testis in some animals and as an ultrafiltrate in others. The suspension was prepared by grinding approximately 1 gm. of testis, 2 cc. of 0.85% saline solution, and sand with a sterile mortar and pestle. The filtrates were made by passing the suspension diluted 1:2 with saline through a No. 3 Seitz filter.

The alterations of the synovial membrane were of two types: (*a*) Trauma was induced by scratching the synovial membrane three times with a 20 gauge needle. Analogous procedure in the skin resulted in a positive Calmette-Guerin reaction in two of three animals receiving

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vaccinia virus filtrate by vein. The phenomenon could not be elicted when the testicular route was employed. (*b*) Introduction of several growth products of Group A, beta hemolytic Streptococci was carried out. Dr. Alan Bernheimer kindly prepared solutions of streptolysin O and streptolysin S. To serve as controls for these, the solutions were inactivated with heat so that their hemolytic activity was less than 1% of the original materials. Similarly, tests were carried out with streptokinase-streptodornase (Lederle) and with solutions of this material inactivated by being placed in a bath of boiling water for one hour.

TABLE 2.—*Effect of Trauma on Experimental Viral Lesions of Joints of Rabbit* *

Experiment No.	Procedure: Right Knee	Procedure: Left Knee	Procedure: Extra-articular Virus	Day Killed	Postmortem Findings: Heart	Postmortem Findings: Right Knee	Postmortem Findings: Left Knee	Postmortem Findings: Misc.
14	T	T	—	5	++ my	± nonspecific sy	± nonspecific sy	—
15	T	—	V s, t	4	—	+ nonspecific sy	+ sy	Neg. C. G.
16	T	—	V f, 0.3 cc., v	5	+ my	± nonspecific sy	+ nonspecific sy	Pos. C. G.
17	T	—	V f, 0.3 cc., v	4	—	+ sy	+ nonspecific sy	Pos. C. G.
18	T	—	V f, 0.6 cc., v	4	—	+ nonspecific sy	± nonspecific sy	Neg. C. G.
19	T	—	M s, t	7	+ my	± nonspecific sy	± nonspecific sy	—
20	T	—	Virus III, t	3	+ my	± nonspecific sy	Normal	—

* Key to symbols: T, traumatized with needle; V, vaccinia; s, suspension; t, intratesticular; f, filtrate; v, intravenous; M, myxoma; my, myocarditis; sy, synovitis; C. G., Calmette-Guerin.

TABLE 3.—*Effect of Streptolysin O on Viral Lesions of Joints and Heart of Rabbit* *

Experiment No.	Procedure: Streptolysin O, Right Knee, Units	Procedure: Heat-inactivated Streptolysin O, Left Knee, Units	Procedure: Extra-articular Virus	Day Killed	Postmortem Findings: Heart	Postmortem Findings: Nonspecific sy, Right Knee	Postmortem Findings: Nonspecific sy, Left Knee
21	42	42	—	5	+ my	+	+
22	105	105	—	5	+ to ++ my	±	±
23	210	210	—	5	++ my	±	±
24	210	210	—	5	—	+	+
25	210	210	Va, t	4	—	±	±
26	210	210	Va, t	4	+ v	+	+
27	210	210	Va, t	4	+ v and my	+	+
28	210	210	Va f, ve	5	+ my	+	+
29	210	210	Va f, ve	4	+ my	±	±
30	210	210	Va f, ve	5	—	+	+
31	210	210	Va f, ve	6	++ my	+	+
32	210	210	Myx, t	9	+ my	+	+
33	210	210	Myx, t	11	—	±	—
34	210	210	Myx, t	11	—	±	+

* Key to symbols: my, myocarditis; v, valvulitis; Va, vaccinia; t, intratesticular; f, filtrate; myx, myxoma; ve, intravenous; sy, synovitis.

The joints were inspected and their circumferences measured at daily intervals. Rectal temperatures also were recorded daily. The protocols of the experiments are summarized briefly in Table 1. The animals were killed by injecting air into the veins of the ear. The joints and blocks of the viscera were fixed in Zenker's solution. Two sections of each knee were made.

RESULTS

Intra-articular inoculation of the three viruses resulted in the development of arthritis. These lesions were quite similar in their morphological and clinical character to those produced by the same preparations when introduced into the testis or

skin. The severity of the lesions was graded from 0 to 4+. Those of 1+ intensity were only of microscopic proportion. Four-plus lesions involved massive swelling of the parts. Equivocal changes have been recorded as ±.

Within 48 hours of the inoculation of vaccinia virus, the knees became swollen; their landmarks were no longer palpable because of edema of the periarticular structures. Some fever then developed, and the animals became moribund in 96 hours. At this time the thigh was markedly edematous. Section revealed little or no fluid

TABLE 4.—*Effect of Streptolysin S on Viral Lesions of Joints and Heart of Rabbit**

	Procedure				Postmortem Findings		
Experiment No.	S. S, Right Knee, Units	Heat-Inactivated S. S, Left Knee, Units	Extra-articular Virus	Day Killed	Heart	Non-specific sy, Right Knee	Non-specific sy, Left Knee
35	213	213	—	4	—	±	±
36	425	425	—	4	—	±	±
37	425	425	—	4	++ to +++ my	+	±
38	425	425	Va, t	4	—	+	±
39	212	212	Va f, v	5	+ my	±	±
40	425	425	Va f, v	4	++ my	±	—
41	425	425	Va f, v	4	++ my	+	±

* Key to symbols: S. S, streptolysin S; Va, vaccinia; t, intratesticular; f, filtrate; v, intravenous; my, myocarditis.

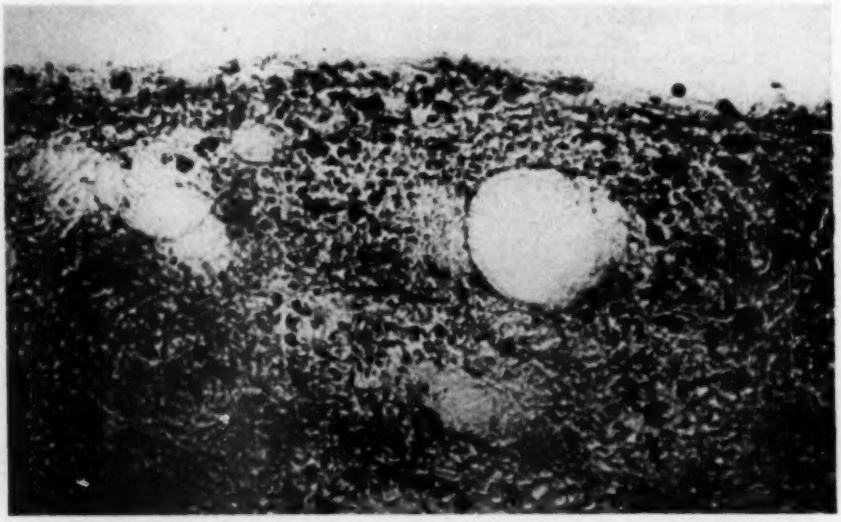

Fig. 1.—Vaccinia virus; the synovial membrane has undergone necrosis; × 280.

to be present within the articular cavity. The capsule and periarticular structures (including subcutaneous tissues and striated muscle) were distended with fibrin-containing edema fluid. The synovial membrane had become necrotic in many areas, as had a small proportion of the superficial chondrocytes of the articular cartilage. Large numbers of polymorphonuclear leucocytes and mononuclear cells infiltrated the synovial membrane (Fig. 1). There was considerable exudation of fibrin into it. Inclusion bodies could not be demonstrated. In Table 1, synovitis has been classified

as nonspecific unless it was accompanied by necrosis. Focal coagulation necrosis also was observed in the marrow and trabeculae of the diaphysis subjacent to the epiphyseal plate when the virus had been injected intravenously.

The articular swelling induced by the virus of infectious myxoma appeared in five to six days. It was of milder degree than in the case of vaccinia virus. The disease ran its typical fatal course in several days, with development of conjunctivitis and rhinitis. Moderate amounts of gelatinous exudate were present within the affected articular cavities. The synovial membranes and capsules were thickened by edema and had a mucoid consistence. Microscopically, distension of these structures by fibrin-containing fluid was the most conspicuous change. Moderate numbers of mononuclear cells and a smaller proportion of polymorphonuclear leucocytes also were present, as well as the large scattered stellate cells characteristic of this disease

TABLE 5.—*Effect of Streptokinase-Streptodornase on Viral Lesions of Joints and Heart of Rabbit* *

Experiment No.	Procedure: Right Knee, Units	Procedure: Left Knee	Procedure: Extra-articular Virus	Day Killed	Postmortem Findings: Heart	Postmortem Findings: Right Knee	Postmortem Findings: Left Knee
42	82 SD 100 SK	H SK-SD	—	5	++ my	± n sy	+ n sy
43	328 SD 400 SK	H SK-SD	—	5	—	± n sy	± n sy
44	820 SD 1,000 SK	H SK-SD	—	5	—	—	± n sy
45	82 SD 100 SK	H SK-SD	Va, t	4	—	—	± n sy
46	410 SD 500 SK	H SK-SD	Va, t	5	+ val +++ my	± n sy	—
47	820 SD 1,000 SK	H SK-SD	Va, t	5	—	± n sy	± n sy
48	410 SD 500 SK	H SK-SD	Va, v	5	—	± n sy	± n sy
49	820 SD 1,000 SK	H SK-SD	Va, v	5	++ my	+ n sy	+ n sy

* Key to symbols: H, heat-inactivated; Va, vaccinia; t, intratesticular; v, intravenous; my, myocarditis; val, valvulitis; n, nonspecific; sy, synovitis; SD, streptodornase; SK, streptokinase.

(Fig. 2*A*). Edema also was present in the periarticular tissues, but much less than in the experiments with vaccinia.

Virus III produced no gross alterations in the appearance of the joint or in symptoms of systemic illness. Nevertheless, inoculation of the suspension resulted in the development of synovitis. This was characterized microscopically by infiltration of moderate numbers of mononuclear cells in the synovial membrane. A fair proportion of these had large characteristic intranuclear inclusion bodies (Fig. 2*B*). Although there was some proliferation and swelling of the lining cells of the synovial membrane, inclusion bodies were not seen within them.

Only mild nonspecific inflammatory changes were observed when autoclaved preparations of these viruses were instilled into the contralateral knees.

None of the streptococcal growth products investigated gave evidence of potentiating the localization of viruses in the joints. Relatively large amounts of these materials were administered, and the viruses were highly pathogenic. Vaccinia virus, inoculated intravenously, entered and perhaps proliferated in the synovial tissues. This was demonstrated by grinding an aliquot of synovial membrane of the knee

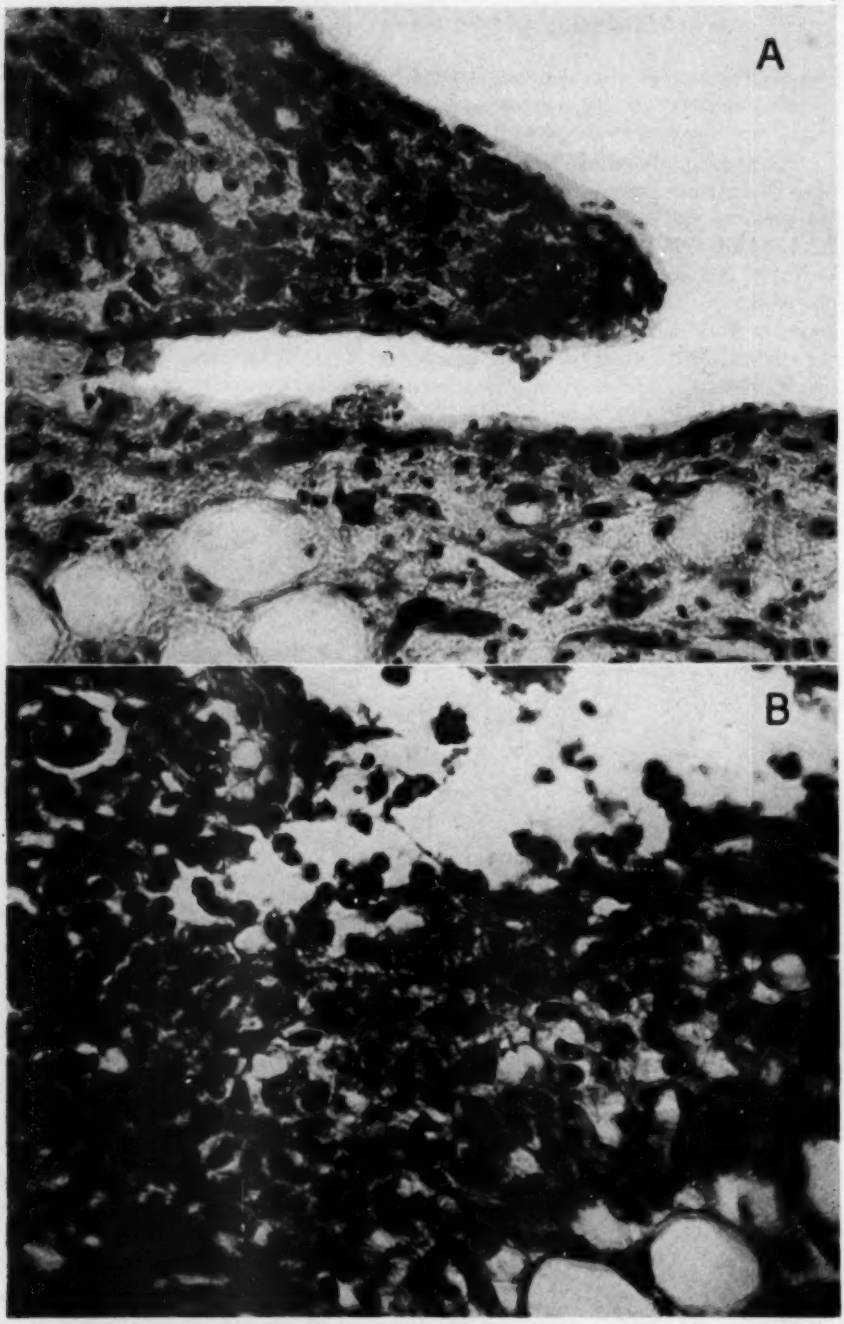

Fig. 2.—*A*, infectious myxoma virus; the synovial membrane is edematous and infiltrated with inflammatory cells. In addition to polymorphonuclear leucocytes, there are the scattered large cells characteristic of this disease. The synovial lining cells are swollen; × 280. *B*, virus III; there is infiltration of mononuclear cells into the synovial membrane. Within some of these, large inclusion bodies are seen; × 280.

with sand in a mortar and pestle. A suspension with 10 parts of infusion broth was centrifuged and forced through a Swinny filter. Serial 10-fold dilutions with broth were made. One-tenth cubic centimeter of each of these was injected into the shaved skin of a young rabbit. A reaction was considered positive if at the end of 72 hours the site was red and indurated over an area at least 5 mm. in diameter. In one of the rabbits receiving intra-articular streptolysin S and heat-inactivated streptolysin S, vaccinia virus was found at dilutions of 10^{-3} in each knee.

Myocarditis occurred with high frequency in this group of rabbits, and we cannot be sure that its incidence or severity was increased as the result of intra-articular administration of the streptococcal preparations. In two of the seven rabbits receiving streptolysin O and vaccinia virus, acute necrotizing mitral valvulitis developed. This may well reflect the intracardial localizing effect of this toxin on viruses described by Pearce. The failure to observe a more severe grade of myocardial change may be due to the fact that the streptolysin O was not introduced directly into the blood stream and so to the heart. A mild degree of valvular inflammation, as well as massive necrosis of the myocardium, developed in one of the rabbits that was treated with streptokinase-streptodornase and vaccinia virus.

COMMENT

The experiments demonstrate that certain filtrable viruses, when entering the articular structures in sufficient quantities, can evoke the development of arthritis. Under the conditions of these experiments none of the growth products of the hemolytic Streptococcus investigated—streptolysin O, streptolysin S, or streptokinase-streptodornase—exerts an appreciable potentiating influence on the development of viral arthritis. The fact that streptolysin O does influence the development of viral carditis in rabbits indicates that this is in some manner a cardiotropic action and does not involve the other structures that are affected in rheumatic fever. If, indeed, rheumatic fever is a viral lesion provoked by a preceding streptococcal infection, the relation between the virus and the bacterium is of a more specific type than the hypothetical one postulated above.

SUMMARY AND CONCLUSIONS

Intra-articular inoculation of vaccinia virus, infectious myxoma virus, and virus III in rabbits results in the development of arthritis. When the viruses are injected intravenously or intratesticularly, specific arthritis does not develop as the result of traumatizing the synovial membrane or exposing it to streptolysin O, streptolysin S, or streptokinase-streptodornase.

MORPHOLOGY OF THE KIDNEY IN MORBUS CAERULEUS

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THE PURPOSE of this paper is to report the histopathological findings in the kidney from patients with morbus caeruleus (the cyanotic group of congenital heart disease, with persisting venous-arterial shunt). These congenital heart malformations with a right to left shunt and cyanosis are considered here under the collective term morbus caeruleus. The reduction of average oxygen tension and the presence of at least 5 to 6 gm. of reduced hemoglobin in 100 cc. of blood are responsible for cyanosis. Fallot's tetralogy, with its variations, predominates in patients with morbus caeruleus. During recent pathological anatomy studies on morbus caeruleus carried out in this institute (Giampalmo and Schoenmackers,[1] Gusmano,[2] Meessen and Stochdorph,[3] Schoenmackers [4]), it seemed of interest to examine the kidney for morphological changes resulting from the chronic hypoxia.

Glaser and co-workers [5] have reported that the oxygen requirement of the kidney per unit of weight is more than that of most other organs. According to Rein,[6] the blood flow to the kidneys can amount to 30% of the cardiac output. Moreover, the kidneys predominate over all other organs in respect to the relation of their share of basal metabolism ($1/12$) to their share of body weight ($1/250$). We have not found any pathologicoanatomical investigations of the morbus caeruleus kidney in the literature available to us.

MATERIALS AND METHODS

Twenty-eight kidneys were selected from patients with morbus caeruleus. The ages varied from 3 months to 28 years. The heart malformation, compensatory vascularization of the lungs, and the clinical data are given in the Table. The average number of glomeruli counted

From the Department of Pathology (Director Prof. Dr. Meessen), Medical Academy.

1. Giampalmo, A., and Schoenmackers, J.: Die Lunge bei Morbus caeruleus, Beitr. path. Anat. **112**:387, 1952.

2. Gusmano, G.: Die Leber bei Morbus caeruleus, Frankfurt. Ztschr. Path., to be published.

3. Meessen, H., and Stochdorph, O.: Gehirnbefunde bei Morbus caeruleus, to be published.

4. Schoenmackers, J.: Pathologisch-anatomische Befunde bei Morbus caeruleus, Verhandl. deutsch. Gesellsch. Kreislaufforsch. **16**:179, 1950.

5. Glaser, H.; Laszlo, D. and Schürmeyer, A.: Über den Energieumsatz der Niere, Arch. exper. Path. u. Pharmakol. **168**:139, 1932.

6. Rein, H.: Einführung in die Physiologie des Menschen, Ed. 10, Berlin, Springer-Verlag, 1949, 241.

Data on Morbus Caeruleus Cases and Control Cases

Case No.	Age, Yr.	Sex	Malformation of Heart: Tricuspid Atresia	Infundibulum Stenosis	Pulmonary Stenosis	Auricular Septal Defect	Ventricular Septal Defect	Patent Ductus Arteriosus	Pulmonary Compensation *	Degree of Cyanosis	Hb †	RBC,‡ Million	O_2 Deficit, Vol. %	O_2 Capacity, Vol. %	O_2 Content, Vol. %	Glomerular Count
1	¼	♂	..	..	+	..	+	..	+	+	....	....	....	....	....	30
2	1¾	♀	..	..	..	..	+	..	..	..	....	....	....	....	....	19
3	2⅝	♀	..	..	+	+	+	+	+	++	....	....	....	....	....	20
4	3	♀	..	Subtotal +		+	+	..	±	+	146	8.0	45.0	28.0	15.4	14
5	3¾	♀	..	+	..	Subtotal +		§	+	++	....	....	....	....	....	11
6	4	♂	..	..	+	+	+	..	++	+	....	....	....	....	....	17
7	4	♀	..	..	..	..	..	+	..	..	....	....	....	....	....	10
8	4	♂	..	..	+	+	+	+	..	++	140	8.4	37.9	29.9	15.6	10
9	5	♂	+	..	..	+	+	+	±	++	147	7.4	47.0	28.8	17.0	12
10	5	♂	..	..	+	..	+	+	..	+	150	7.4	....	....	....	13
11	5	♀	..	+	+	..	+	+	±	+	153	8.3	....	....	....	11
12	5½	♂	..	+	..	..	+	..	..	..	....	....	....	....	....	18
13	6	♂	..	..	+	..	+	+	++	+	144	8.6	27.1	27.3	19.9	15
14	6	♀	..	+	..	..	±	+	±	++	160	7.8	....	....	....	16
15	7	♀	..	..	+	..	+	..	+++	++	153	6.8	....	....	....	9
16	9	♀	..	..	..	+	+	..	..	..	110	5.4	28.1	....	....	8
17	10	♂	..	+	..	+	+	..	+	+++	160	8.4	42.0	34.2	20.2	8
18	10	♀	..	..	+	+	+	..	..	+	120	6.1	....	....	....	9
19	12	♀	+	..	+	..	+	+	+	+++	....	....	....	....	....	11
20	13	♂	..	..	+	+	+	‖	..	+	....	....	....	....	....	10
21	13	♂	+	..	+	+	+	+	+++	+	145	7.2	35.0	....	20.0	10
22	15	♂	..	..	+	+	+	..	+	+	160	8.3	31.5	30.8	21.1	10
23	15	♂	..	+	..	+	+	..	++	++	160	9.2	69.0	29.5	9.0	8
24	15	♂	..	+	..	+	+	..	..	+++	154	7.8	....	....	....	9
25	16	♂	..	..	+	+	+	+	..	++	152	7.8	....	....	....	6
26	18	♀	..	..	+	..	..	+	..	+	....	....	....	....	....	8
27	18	♂	..	..	+	..	+	..	+	+	....	....	....	....	....	10
28	21	♀	..	+	..	..	+	..	+	+	....	....	....	....	....	7

Control Group

			Principal Disease	Cause of Death	
29	1 day	♀	Premature birth	Aspiration	32
30	⅝	♂	Whooping cough	Intracranial pressure	13
31	1⅛	♂	Fracture of skull	Cerebral contusion	16
32	1½	♀	Myocarditis	Cardiac failure	13
33	2¾	♂	Poliomyelitis	Respiratory paralysis	15
34	4½	♂	Measles	Encephalitis	14
35	9½	♂	Pinealoma	Intracranial pressure	10
36	10½	♀	Meningeal tuberculosis	Intracranial pressure	9
37	15	♂	Spongioblastic glioma	Intracranial pressure	6
38	16	♂	Purulent ethmoiditis	Meningitis	9
39	19	♂	Poliomyelitis	Cardial failure	7
40	19	♂	Meningeal tuberculosis	Intracranial pressure	7
41	20	♀	Endometritis	Air embolism	6
42	24	♂	Silicosis	Cardiac failure	6

* Compensatory vascularization of lungs from axillary, mediastinal, mesopulmonary and dilated bronchial arteries.
† Hemoglobin.
‡ Red blood cells.
§ Cor biloculare, hypoplasia of pulmonary artery.
‖ Patent foramen ovale, dextroposition of aorta.

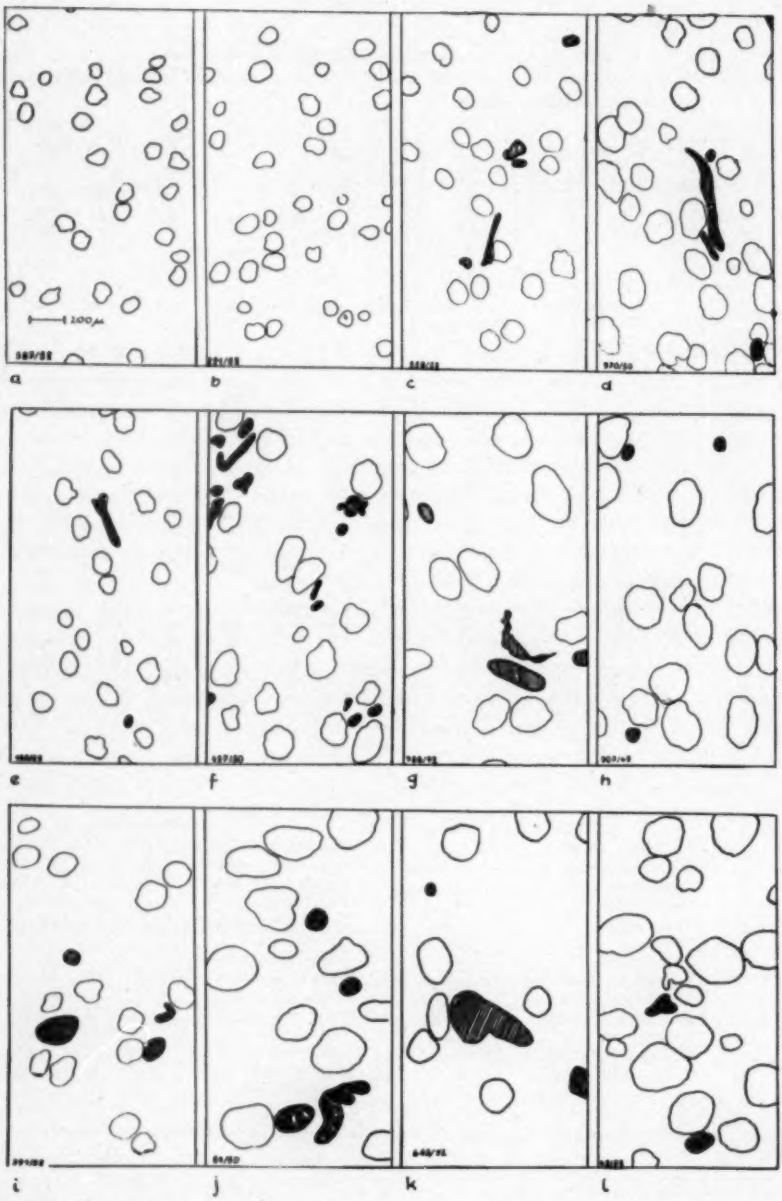

Fig. 1.—Size of glomeruli in normal controls (*a, c, e, i, k,*) and in morbus caeruleus patients (*b, d, f, g, h, j, l,*), depending on age.

in five fields (magnification × 60) are given in the Table. A control group of 14 kidneys from patients in the same age groups was examined for comparison. Age, principle disease, and cause of death are also given in the Table.

Frozen sections and sections embedded in paraffin of formalin-fixed kidneys were examined microscopically. Tissue sections were cut at 5 to 6 μ. The following histological techniques were employed routinely on both pathological and normal specimens: hematoxylin and eosin, Van Gieson's elastic tissue stain, Masson's trichrome stain, and periodic acid Schiff stain. Kidney architecture and the number and size of glomeruli were examined by projecting 5 to 6 μ specimens on drawing paper and tracing the outlines of Bowman's capsule of at least 100 glomeruli. Figure 1 shows sections of the drawings. The diameters of Bowman's capsule in Case 9 and Case 28 were measured with an ocular micrometer and compared with corresponding control cases. In Case 9, a boy, age 5 years, values ranged from 254 to 275 μ, normal value 149 μ. In Case 28, a woman, age 21 years, values ranged rom 256 to 315 μ, normal 189 to 213 μ.

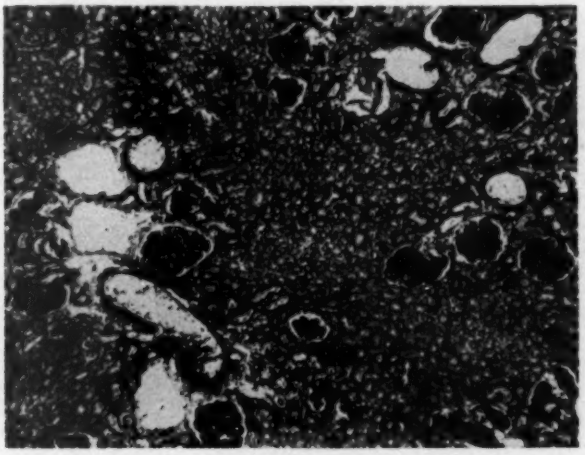

Fig. 2. (Case 10).—Lake-like dilated veins in the boundary zone. Masson's trichrome stain; × 25.

Apart from cyanosis and congestion, the macroscopical appearences of the morbus caeruleus kidneys were inconspicuous. The microscopical findings of six representative kidneys from patients of different age groups are summarized.

Case 4.—Kidney structure was in general well preserved. There were large glomeruli in the cortex which appeared densely distributed (Fig. 1*d*). Capillary loops of the glomeruli often filled the capsular space. An aneurysmatic widening of the capillary loops was often seen. There was neither thickening of the basement membrane nor increase in number of mesangium cells. Intertubular capillaries in the cortex and particularly in the medulla showed congestion. Walls of arterioles were normal. Vasa afferentia were very wide and gaping. Large arteries showed a normal wall structure. No increase in cell number of the juxtaglomerular apparatus was evident. The veins were markedly wide and gaping. The tubular epithelium was intact.

Case 10.—Kidney structure was normal. Vessels in the boundary zone were cut transversely. The arcuate arteries and veins and the interlobular arteries and accompanying veins were very wide (Fig. 2). The glomeruli in the cortex were

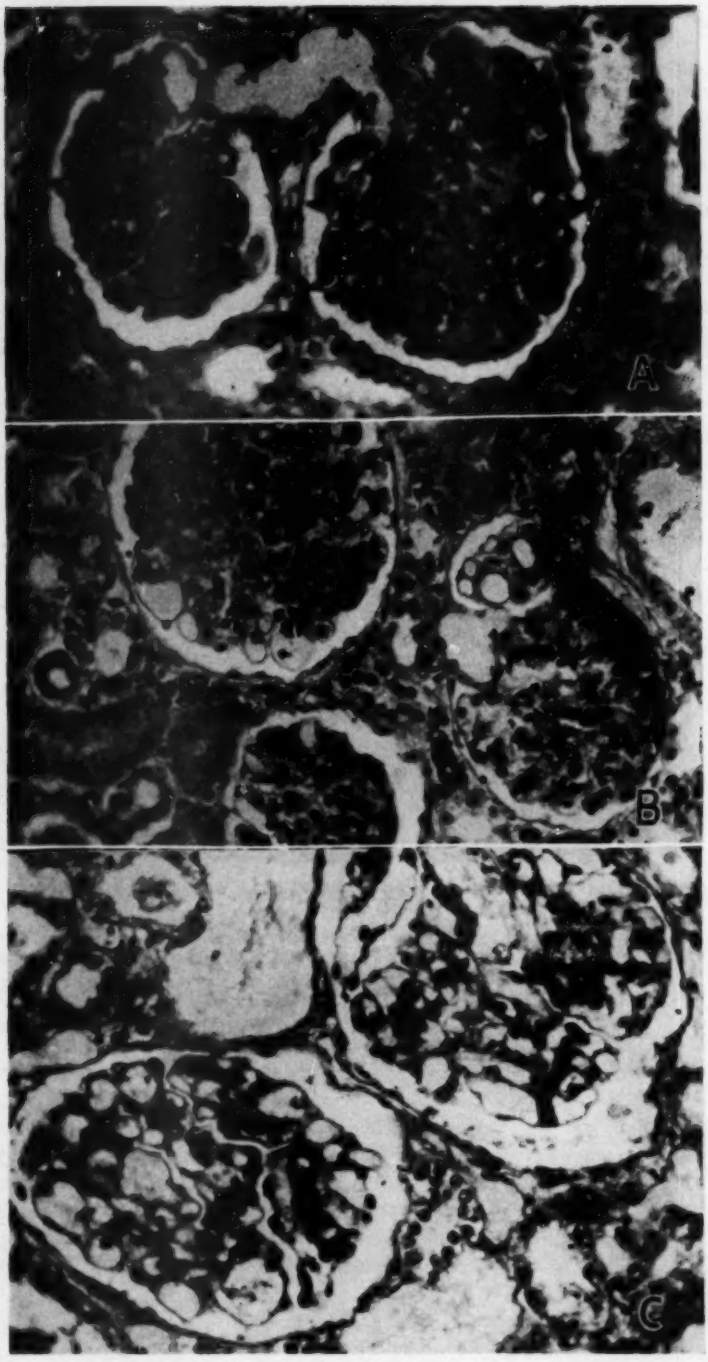

Fig. 3.—*A*, widened vasa afferentia of two glomeruli (Case 13). Masson's trichrome stain; × 250. *B*, aneurysmatic capillary loop ectasia in two glomeruli (Case 13). Masson's trichrome stain; × 250. *C*, enlarged glomeruli with capillary loop ectasia and delicate mesangium (Case 17). Masson's trichrome stain; × 450.

enlarged, the capillary loops congested. A few loops showed aneurysmatic widening. Vasa afferentia were widened; vasa efferentia appeared constricted. Tubular epithelium and interstitial tissue were intact.

CASE 13.—Kidney structure was in general well preserved. Glomeruli were large; capillary loops were partly empty and gaping, partly congested. Figure 3*A* shows the lake-like widened vasa afferentia of two glomeruli. Capillary loop ectasia was evident in the empty loops (Fig. 3*B*). A few glomeruli in the cortex showed complete fibrous obliteration; others showed partial obliteration of the loops. Figure 4 shows a glomerulus in which the capillary loops are partly obliterated, with only slight increase of cells. The interstitial tissue often showed fibrotic enlargement, the distribution of the glomeruli appearing less dense (Fig. 1*g*).

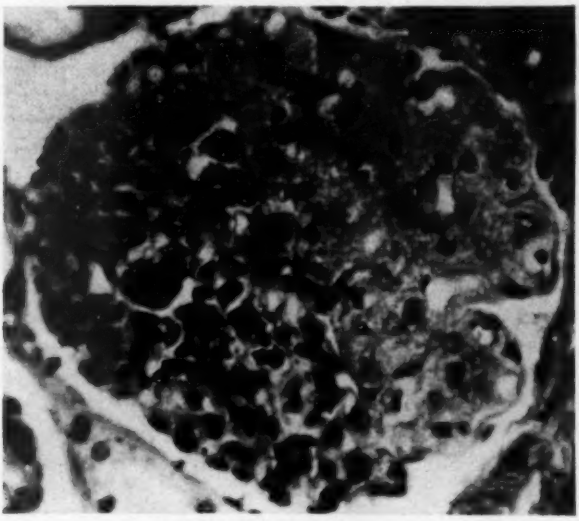

Fig. 4 (Case 13).—Glomerulus showing partial obliteration and partial congestion. Masson's trichrome stain; × 450.

CASE 17.—Capillary loop ectasia of the enlarged glomeruli was particularly marked (Fig. 3*C*). The vasa afferentia, intertubular capillaries, and veins were very wide. No other changes were evident.

CASE 25.—Nearly all glomeruli in the cortex showed extreme enlargement and capillary loop ectasia. A few glomeruli showed fibrotic obliteration; the interstitium was widened, and slight cellular infiltration was evident. The tubules were intact; a few areas showed protein-like masses in the tubules. The periarteriolar pad was not evident on the widened vasa afferentia.

CASE 28.—Serial section showed a triangular fibrous scar with the base near the capsule and therein a group of hyalinized glomeruli. An artery with intimal thickening and partial occlusion of the lumen was evident. In the boundary zone the glomeruli were very large and densely distributed; the capillary loops were wide and congested. Tubular epithelium was intact. Arteries and veins showed a normal wall structure.

FINDINGS

The correlation between age and grade of morbus caeruleus and glomerular enlargement is evident from the above data. This becomes striking on comparison of glomerular architecture of morbus caeruleus patients and that of normal controls (Fig. 1). In the age group 1 to 2 years there is only a slight difference between the normal control patients (Fig. 1*a*) and those with morbus caeruleus (Fig. 1*b*). The difference is obvious in the age group 2 to 3 years (Fig. 1*c* and *d*). In Case 4, the pulmonary stenosis was subtotal. The enlargement of the glomeruli resulting from capillary loop ectasia was particularly marked (Fig. 1*d*). The glomerular enlargement of 4, 6, and 7-year-old patients with morbus caeruleus is impressive in comparison with a 4½-year-old control patient (Fig. 1*e*). Figure 1*i*, *j*, *k*, and *l* shows the findings in 16 and 20-year-old patients with morbus caeruleus and those in normal controls.

In consideration of the data, we believe that glomerular enlargement resulting from capillary loop ectasia is a characteristic of the morbus caeruleus kidney. The grade of glomerular enlargement varies but depends obviously on the grade of cyanosis. However, a strict parallelism between anatomical findings and clinical data was not observed. Widening of the vasa afferentia was often observed. In our opinion this arteriolar widening is a prerequisite for the increase in glomerular circulation. In contrast, the vasa efferentia were seldom widened, and the wall structure was normal. The juxtaglomerular apparatus of the vas afferens was reduced, and the cells of the periarteriolar pad were in most cases hardly definable.

The kidney architecture was altered in respect to the widening of the larger vessels, particularly the veins. The walls of the veins appeared thin, but histological changes were not evident. The varied congestion of the intertubular capillaries probably depends upon agonal processes. A marked congestion of the *Bueschelvenen* in the medulla was evident in each kidney. The glomerular counts of morbus caeruleus kidney and those of control patients show no essential differences. The dense distribution of glomeruli in morbus caeruleus is a result of glomerular enlargement.

The remaining histological findings of the morbus caeruleus kidney are not characteristic. Fibrous obliteration of glomeruli is attributable to thromboembolic processes due to the fact that in many of the morbus caeruleus cases an endocarditis was present. The widening of the interstitial tissue was observed, as a rule, only when glomerular obliteration and atrophy of the tubules were present. Otherwise the tubules were intact. No casts or fatty degeneration of the tubular epithelium were observed.

For the interpretation of these results, we believe that the chronic hypoxia and the increase of carbon dioxide are prime factors. Consequent dilation of the glomerular arterioles and widening of the vasa afferentia facilitate the glomerular circulation. The adaptation to the chronic hypoxia is accomplished by capillary loop ectasia, which results in glomerular enlargement. These local tissue adaptations in the kidney and the developing polycythemia fully compensate for the chronic hypoxia. Thus no damage due to oxygen deficiency results. The tubular epithelium was always found intact. The gradually developing hypoxia differs from the acute oxygen deficiency in the kidney similarly to that in the brain, as demonstrated by Meessen and Stochdorph.[3]

Scott and Elliot [7] have investigated the possible effects of anoxemia and polycythemia on renal hemodynamics and excretory functions in 19 patients with congenital malformations of the heart and cyanosis. The authors report that the glomerular filtration rates are normal despite depressed renal plasma flow. The renal blood flows were above the normal standard in most of the patients. These investigations were carried out by measurements of paraaminohippuric acid and inulin clearances. We are of the opinion that these clinical findings are correlative with our anatomical findings of glomerular enlargement and resulting increase of available surface for filtration. There, too, the widening of the vasa afferentia would facilitate the glomerular circulation. We found no specific anatomical evidence to support the presumed increase in postglomerular resistance (Scott and Elliot [7]).

The kidney of patients with morbus caeruleus is clearly differentiated from the acute and chronic congestive kidney. These conditions are manifest chiefly in the venous part of the kidney. An agonal congestion is, however, often found in morbus caeruleus kidneys. It will be of interest to examine the kidney for similar compensatory mechanisms in other malformations of the heart and great vessels and in chronic valvular disease.

SUMMARY AND CONCLUSIONS

The histological findings in the kidney from 28 patients with morbus caeruleus are reported.

Capillary loop ectasia with resulting glomerular enlargement is characteristic of the morbus caeruleus kidney. Vasa afferentia are widened, and the draining veins are dilated.

Fibrous obliteration of glomeruli, partial obliteration of capillary loops, and interstitial tissue widening are described.

The chronic hypoxia of morbus caeruleus does not result in the destruction of kidney parenchyma.

The anatomical findings in the kidney of patients with morbus caeruleus are a prerequisite for the interpretation of clinical function tests of the morbus caeruleus kidney.

Prof. Dr. Derra, Director of the Surgical Clinic, Medical Academy, Düsseldorf, Germany, and Prof. Dr. Grosse-Brockhoff, Clinic of Internal Medicine, University of Bonn, Bonn, Germany, supplied the clinical data.

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7. Scott, H. W., Jr., and Elliot, S. R., II: Renal Hemodynamics in Congenital Heart Disease, Bull. Johns Hopkins Hosp. **86**:58, 1950.

Case Reports

LEONTIASIS OSSEA, SLIPPED EPIPHYSES, AND GRANULOSA CELL TUMOR OF TESTIS WITH RENAL DISEASE

Report of a Case with Autopsy Findings

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LEONTIASIS ossea secondary to renal disease and granulosa cell tumor of the testis are two lesions which hitherto have not been reported. The two lesions occurred in a single patient, a 21-year-old man, who had congenital anomalies of the urinary tract and generalized osteitis fibrosa. This case report provides two additional features: (1) a continuous seven-year record of renal acidosis with relevant studies of the blood chemistry, (2) the development and pathology of slipped epiphyses.

REPORT OF CASE

In May, 1944, at the age of 13½ years, the patient experienced his first known attack of pyelonephritis. This was treated elsewhere and subsided after five days of sulfonamide therapy. Prior to that time he had been well. His growth had been much slower than that of his brother, and he was classified among the lowest 3% of the population on an age-height scale. During the course of the pyelonephritis, his nonprotein nitrogen level was extremely high (see Table for all chemical data), and in August, 1944, a diagnostic study was made. At that time the boy had no complaints, but careful questioning revealed constant polyuria dating back to infancy.

Physical examination showed intermittent hypertension, borderline cardiac enlargement with an aortic systolic murmur, and tortuosity and narrowing of the retinal arteries. The blood pressure ranged from 170 to 132 mm. of mercury systolic and from 110 to 88 mm. of mercury diastolic. The blood pressure could not be obtained in the legs. Generalized disturbance of bony architecture, consisting of thickened trabeculae and widened epiphyseal lines, was evident on the roentgenograms. A cystogram and a retrograde pyelogram demonstrated a large lobulated bladder and megaloureters. Plastic procedures were performed on both ureters to aid drainage.

Until June, 1945, the boy felt well and followed no therapeutic regimen, but at that time he began to limp, and a slipped right femoral capital epiphysis was found. In September, 1945, the left femoral epiphysis also slipped, and a controlled program of therapy was started. At that time a rachitic rosary and enlargement of the wrists and interphalangeal joints became evident. Because of the impaired ureteral drainage and the elevated value for serum phosphorus, complete bed rest was deemed inadvisable, and walking with crutches was allowed. Medical therapy included a low-protein diet, aluminum hydroxide (3 to 9 oz., 85.05 to 255.5 gm. per day), calcium lactate (4 gm. per day), vitamin D (3,500 units per day), sodium chloride tablets (3 gm. per day), and molar citric acid-sodium citrate mixture (in varied daily dosage, mostly at the 30/15 cc. level). The improvement in the acidosis was striking and was reflected in the patient's feeling of well-being. However, the epiphyseal lesions became worse. A prolonged period of bed rest supplemented by physical therapy was then tried, but slow progression of the bony changes and coxa vara deformity continued.

This study was aided by a grant from the Playtex Park Research Institute.

From the Departments of Pathology and Orthopedic Surgery, Children's Medical Center, and Harvard Medical School.

The asymptomatic infection in the urinary tract, evidenced by slight pyuria, was consistently present and was complicated in May, 1946, by acute orchitis, which quickly subsided after streptomycin therapy. At that time the boy was 16 years old and showed normal adult secondary sex characteristics but developed bilateral gynecomastia. He stopped growing at a height of 155 cm. (61 in.) and was fairly well during the next year, going to school on crutches and driving a motorcycle. In May, 1947, slipping of both proximal humeral epiphyses was noted (Fig. 1*A*) and was attributed to the use of crutches. Canadian crutches were prescribed since it was felt that further changes at the hips should be minimized, even at the expense of humeral deformity. The only revision in medical therapy was a change to a low-phosphorus diet.

Early in 1948, closure of the femoral epiphyses occurred; the crutches were discarded, and the coxa vara showed little progression (Fig. 1*B*). However, at that time the facial bones began to enlarge slowly and painlessly. During the summer he felt well and was able to do some light work and to drive his motorcycle. Bilateral Colles' fractures followed minimal trauma and healed rapidly with some deformity on the right side. The facial deformity, increasing slowly, now obviously involved the mandible, causing enlargement in all directions.

Pertinent Chemical Determinations *

Date	Hemoglobin, Gm./100 Cc.	Nonprotein Nitrogen, Mg./100 Cc.	pH	Carbon Dioxide, mEq.	Protein, Gm./100 Cc.	Sodium, mEq.	Chlorides, mEq.	Potassium, mEq.	Calcium, Mg./100 Cc.	Phosphorus, Mg./100 Cc.	Alkaline Phosphatase, Bodansky Units
May, 1944	....	105	....	....	...	...	...	...	...	...	..
Aug., 1944	13.0	81	....	....	7.6	...	102	...	7.6	7.15	16
June, 1945	7.0	125	7.23	15.1	7.4	...	...	...	7.1	6.0	..
Oct., 1945	9.4	153	7.26	21.8	7.4	141	101	...	8.9	6.1	27
March, 1946	9.6	148	....	20.3	7.0	...	96	...	8.8	6.1	24
Nov., 1946	9.5	116	7.37	....	6.9	...	...	...	8.0	5.8	48
April, 1947	8.5	122	7.33	25.0	6.8	142	96	...	8.5	7.7	58
Nov., 1947	7.7	109	7.33	26.8	7.8	144	98	...	9.2	6.3	56
April, 1948†	10.4	109	7.33	22.1	6.8	138	95	5.3	9.3	4.8	58
Oct., 1948	11.8	87	7.38	24.2	7.4	149	99	5.1	9.8	5.9	55
Oct., 1949	10.2	125	7.25	20.0	6.8	147	97	4.75	9.3	5.7	68
Feb., 1950	9.0	125	7.33	22.0	...	...	97	...	7.8	8.7	72
April, 1951	8.1	166	7.34	19.7	5.9	137	92	5.5	5.8	8.9	30
June, 1951‡	8.7	144	7.3	22.3	5.4	137	86	4.5	5.1	...	21
Sept., 1951	7.8	250	7.26	22.9	5.7	132	74	5.1	4.0	18.4	12

* All values represent medians of determinations during the month listed.
† Albumin-globulin ratio, 4.4:2.5.
‡ Hamilton-Schwartz positive, Ellsworth-Howard negative.

In 1949, additional fractures (clavicle, rib, metacarpals) occurred after minimal trauma and healed rapidly. His general condition did not change much, and he was able to continue in school until April, 1951. His medical regimen during this period was as follows: a daily diet containing 0.3 gm. of calcium and 0.9 gm. of phosphorus, vitamin D (20,000 units per day), calcium lactate (0.8 gm. Ca per day), two glasses of milk (0.6 gm. Ca per day), and molar citric acid/sodium citrate mixture (approximately 45 cc. = 45 mEq. Na per day). The citrate mixture was not well tolerated and was revised from time to time by substituting potassium citrate and acetate in varying amounts to yield 1 mEq. of base per cubic centimeter at a level of 75 to 120 mEq. per day. In 1951, a severe reactive depression developed which was directed at his facial deformity; this was becoming more and more unsightly (Fig. 2). The degree of hypertrophy of the mandible and maxilla was such that each tooth was separated from its fellows by ⅛ in. (0.32 cm.) or more, and, instead of dental occlusion, the hypertrophied body of the mandible impinged on the maxilla behind the molars, causing ulcerations of the overlying mucous membranes. The progression of bony changes was generalized; there was thickening of the calvarium (Fig. 3*A*), an increase in the coxa vara, and intrapelvic protrusion of the acetabula. Radiation therapy to the face was given as a trial measure, with some subjective benefit.

In May, 1951, the boy's general condition declined sharply. He suffered many attacks of epistaxis, and his headaches, formerly mild and transient, became severe. He began to have

intense precordial pain, and his blood pressure rose to 190 mm. of mercury systolic and 130 mm. of mercury diastolic. Intermittent claudication and weakness of the lower extremities reduced him to nearly complete inactivity. Calcification of the arterial walls became evident on roentgenograms and was present in the arteries of the hands (Fig. 3*B*) and feet, as well as in the larger vessels. Significant changes in the blood chemistry were evident. In September, 1951, he contracted epidemic parotitis, which was followed by suppurative staphylococcal parotitis, and he died in uremia.

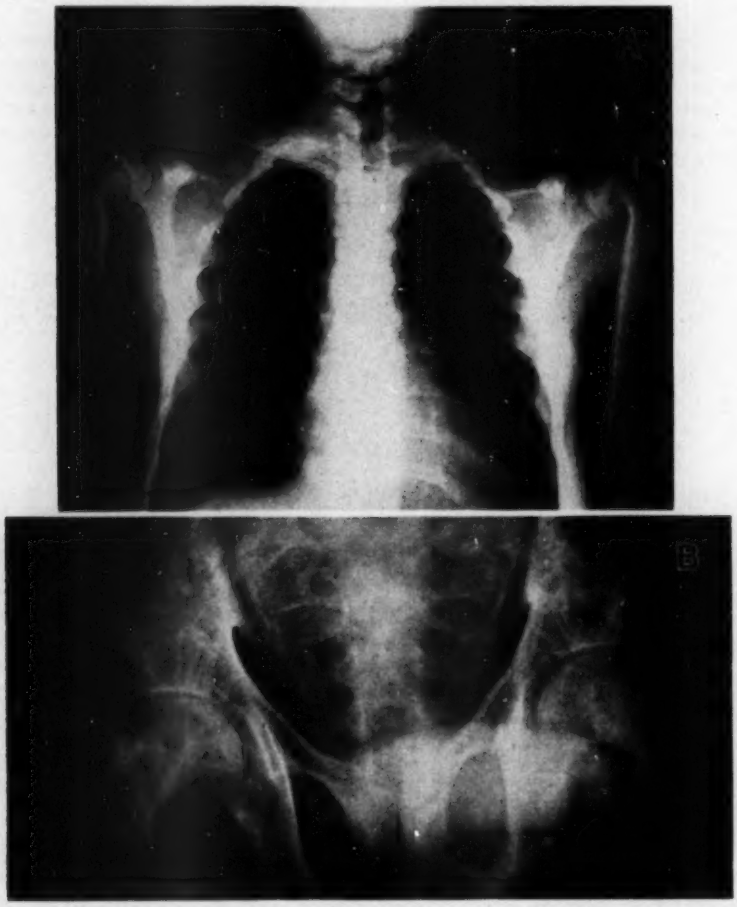

Fig. 1.—*A*, roentgenogram of chest (July, 1947) illustrating slipped humoral epiphyses. *B*, roentgenogram of hips (January, 1948) illustrating slipped epiphyses, beginning acetabular protrusion, and changes in trabecular structure.

Pathologic Observations.—The skeleton showed the following gross abnormalities: The calvarium was increased to about four times the normal thickness, measuring 2 to 3 cm., and was composed of gritty bone of homogeneous texture which cut easily with a knife. It was vascular and adherent to galea and dura. The air sinuses and nasal passages were obliterated, but the foramina were not encroached upon. Incidental osteomyelitis of the left petrous bone and purulent otitis media on the left side, yielding Staphylococcus aureus hemolyticus on culture,

were present. The ossicles on the right side were normal. The maxilla and mandible were massively enlarged and were composed of gritty hypertrophic bone, similar to that found in the calvarium. The teeth were slightly loose but were in good condition. The vertebral column showed mild s-shaped scoliosis (15 degrees) and minimal dorsolumbar kyphosis without wedging of the vertebral bodies but with exaggerated concavity superiorly and inferiorly. The bodies were markedly attentuated in trabecular structure, and the marrow could be scooped out with the finger. The ribs showed considerable enlargement and distortion of the costochondral junctions, a pronounced Harrison's groove, indentation by tortuous enlarged intercostal vessels, and attenuation of the trabecular structure similar to that in the vertebrae. The long bones examined included the proximal portions of both femurs and the right humerus. The head of the humerus showed severe degeneration of the articular cartilage except in that portion in contact with the glenoid fossa, that is, the upper half. The glenoid fossa showed no degeneration of the cartilage. There was about a 30-degree varus deformity of the humeral head on the shaft. A coronal section demonstrated several segments of epiphyseal cartilage remaining at the junction of metaphysis and epiphysis, and this region had changed its appearance from the normal trabeculated structure

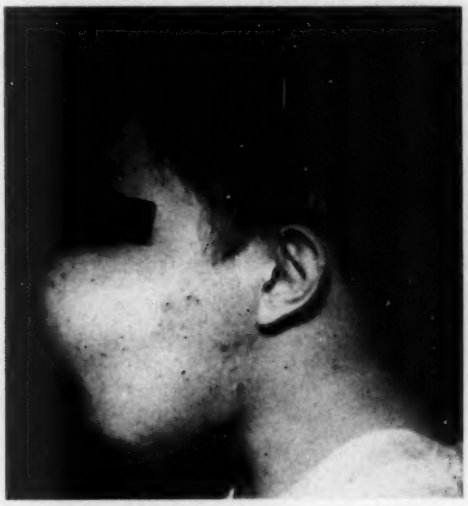

Fig. 2.—Photograph of patient in 1948.

to a gritty amorphous configuration, with a few cysts underlying the degenerated articular cartilage. Those portions of the femurs and pelvis which were examined showed the same conversion of cortical and trabecular architecture as was seen in the humerus (Fig. 4). The acetabular portion of the pelvic bones protruded slightly inward (Otto pelvis) and measured twice the normal thickness. It cut easily with a heavy knife. The acetabular and femoral articular cartilage showed considerable degeneration, especially anteriorly; these areas could be indented by slight pressure of the finger. The femoral neck angle measured 90 degrees on each side, and the femoral heads were rotated posteriorly about 40 degrees. Coronal section revealed cystic degeneration in the subarticular region, alteration of the trabecular pattern, and persistence of epiphyseal cartilage as discontinuous pearly grey nodular strips. The synovial membranes showed slight thickening.

Microscopic examination of the bones revealed that compact bone was entirely absent and was replaced by a lacy cancellous bone undergoing resorption in most areas but showing some bone deposition and becoming osteoid in other areas (Figs. 5 and 6). No traces of haversian systems were seen. The normally cancellous areas

showed a similar pattern. The trabeculae were thin and were separated by loose fibrous stroma containing a few nodular hyperplastic hematopoietic foci. Sections of the femoral and humeral heads (Fig. 4) showed an attenuation of trabecular pattern, but, despite this striking loss of bone, the trabecular orientation resembled the

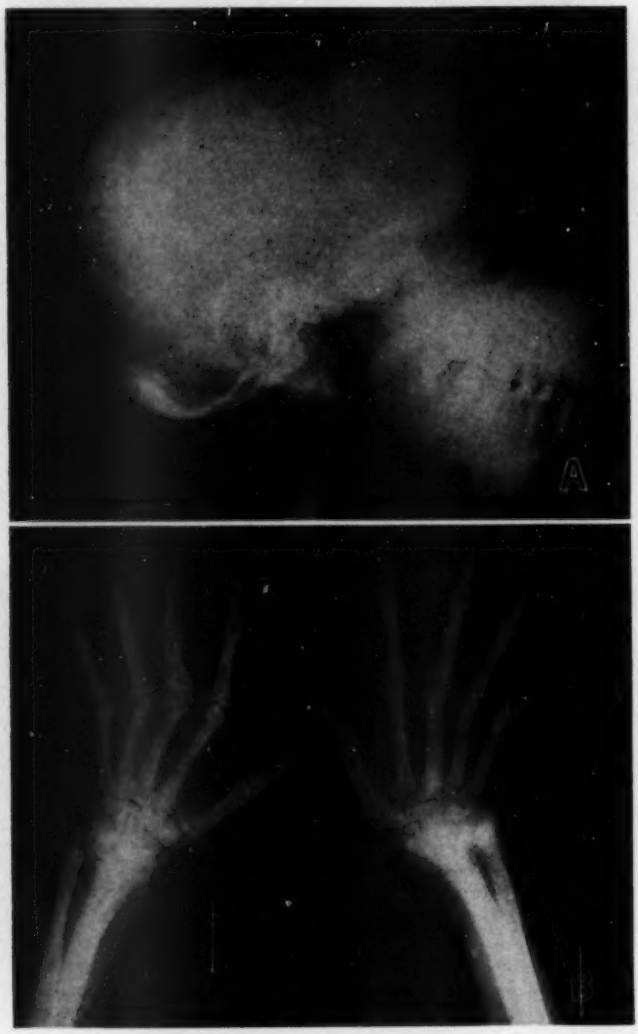

Fig. 3.—*A*, roentgenogram of skull (April, 1951) illustrating severe generalized bony overgrowth, particularly of maxillas and calvarium. *B*, roentgenogram of hands (April, 1951) illustrating calcification of vessels and resorption of terminal phalanges.

normal, except in the area just below the remains of the epiphyseal cartilage. Here an abrupt change in pattern occurred which was accompanied by signs of old hemorrhage denoting the area of slipping. The epiphyseal cartilage was buckled

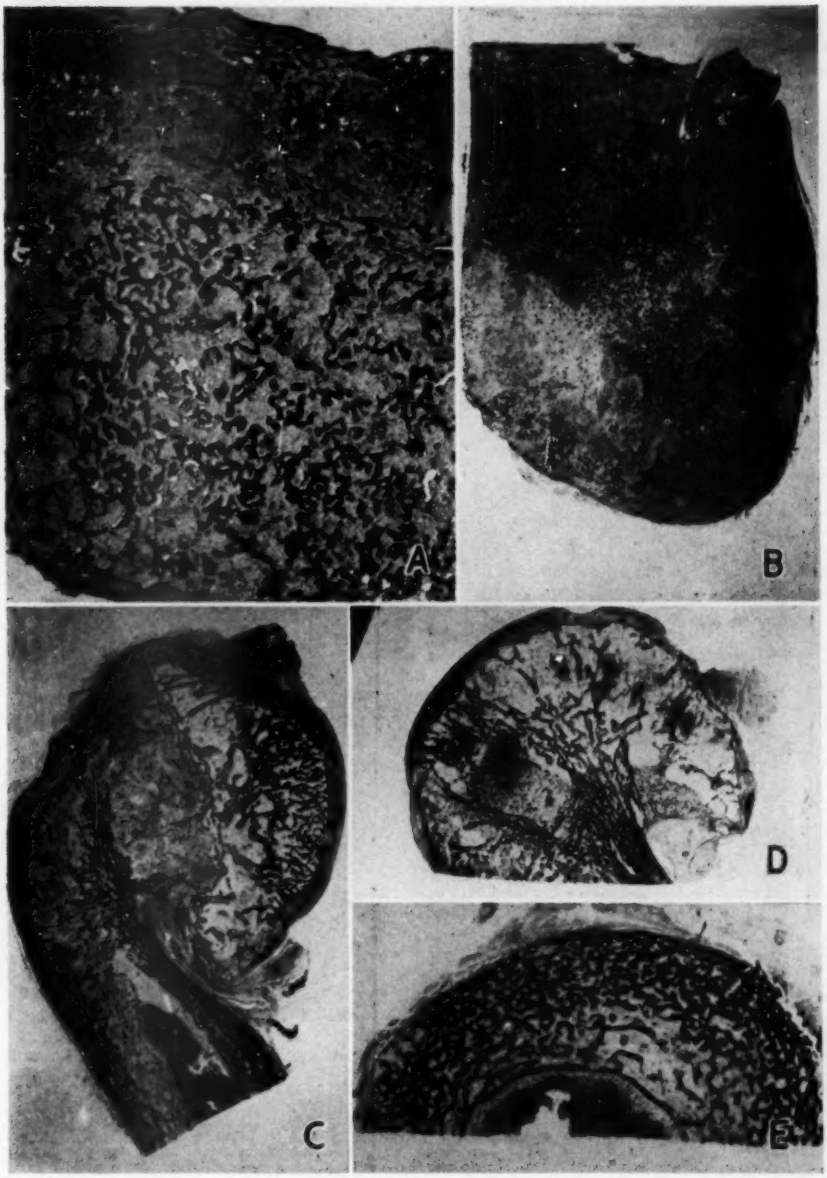

Fig. 4.—Sections of *A*, calvarium (× 11); *B*, mandible (× 2); *C*, humerus (× 2); *D*, femoral head (× 2), and *E*, femoral shaft (× 4) illustrating the fine bony structure. Remains of epiphyseal cartilage can be seen just above the areas of slipping in *C* and *D*.

and fragmented. Each fragment was thicker than normal for the age and showed the sequences of rapid maturation and chondrolysis. The articular cartilage was degenerated in many areas, and in some places the calcified layer showed signs of active resorption.

The heart showed massive left ventricular hypertrophy and dilatation and fibrinous pericarditis. The coronary arteries presented mild intimal thickening. Just

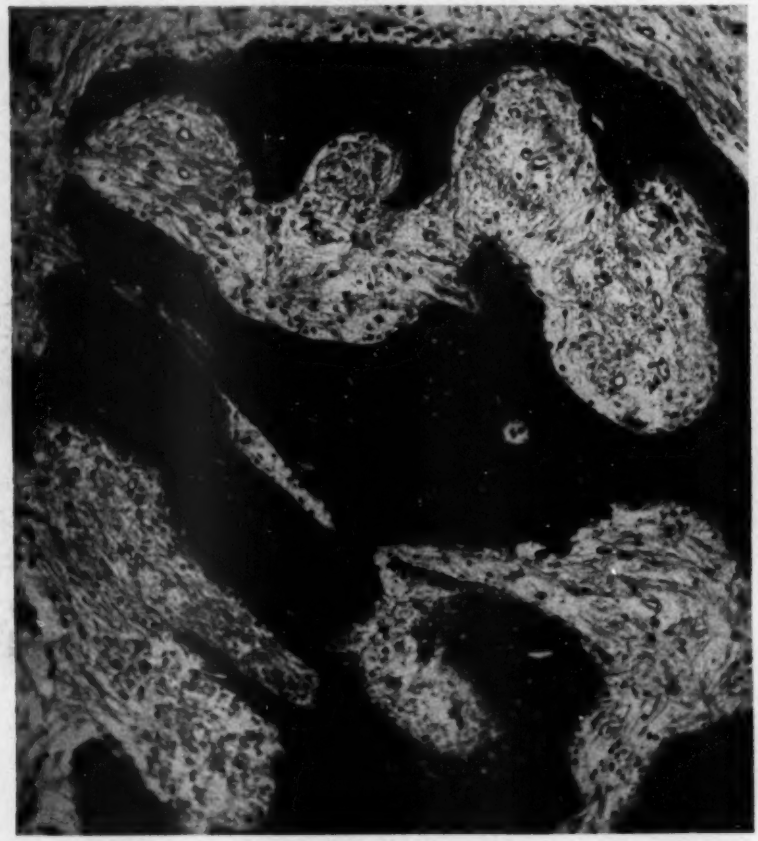

Fig. 5.—Representative area of bone illustrating the diffuse osteitis fibrosa, osteoclasis, and slight osteoid deposition. This pattern predominated in all of the bone sections.

distal to the origin of the subclavian artery and the insertion of an obliterated ductus arteriosus, the aorta was sharply coarcted to an external diameter of 0.7 cm. Above the coarctation the aorta measured 3 cm. in diameter and below, 1.6 cm. At the coarctation site no lumen was present. Collaterals were well developed, and the internal mammary and the deep inferior epigastric arteries were dilated, tortuous, and calcified. Except in vessels, no metastatic calcification was found. The lungs

showed moderate terminal acute congestion and edema. In the spleen, hemosiderosis and extramedullary hematopoiesis were apparent. The liver showed hemosiderosis and mild congestion.

The kidneys were approximately half the normal size. Each weighed 40 gm. and measured 7 by 3 by 2 cm. The perinephric fascia was scarred and was adherent to the thickened capsules. The renal arteries measured only 2 mm. in external diameter. Hydronephrosis was marked, and the renal parenchyma was reduced to a thickness of 1.5 to 2 cm. Corticomedullary demarcation was absent. Microscopically (Fig. 7), the pattern resembled chronic pyelonephritis, with extensive scar-

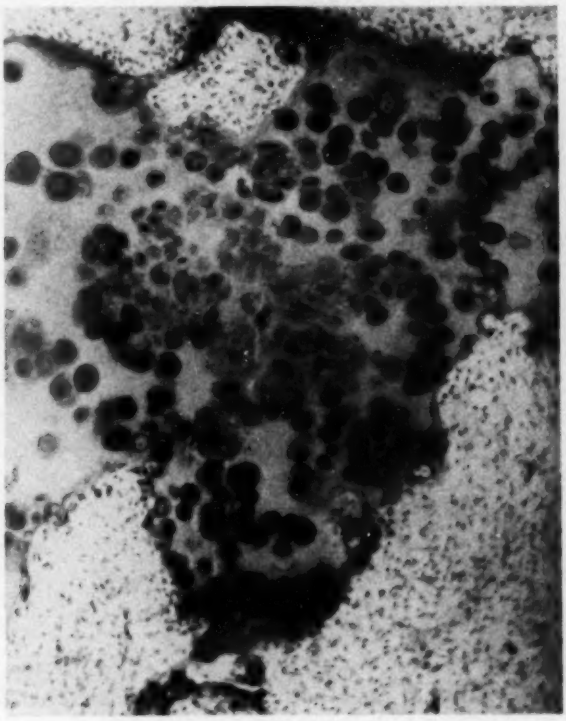

Fig. 6.—Remains of femoral epiphyseal cartilage undergoing chondrolysis. Removal of cartilage matrix by giant cells and invasion of matrix and hypertrophic cells by fibrous marrow is evident. The bone at the top and bottom of the figure represents trabeculae of epiphysis and metaphysis. Note maturation sequences of cartilage directed both toward the epiphysis and toward the diaphysis.

ring and marked reduction in functioning nephrons. Every glomerulus showed some degree of capsular dilatation and capsular scarring. Many of the glomeruli were represented by atrophic tufts lying in a dilated capsular space. The proximal and convoluted tubules lacked differentiation and showed extensive degenerative change. The interstitial tissue was scarred and contained many lymphocytes, a few plasma cells, and histiocytes. The arteries showed elastosis, and the arterioles showed laminated cellular hyperplasia. The ureters were greatly dilated and tortu-

ous and microscopically showed chronic inflammation. The bladder was hypertrophied, and a microscopic survey of its wall revealed only one small collection of ganglion cells. No obstructive lesion of the urinary tract was found.

The right testis was normal in shape and weighed 12 gm. The left testis weighed 8 gm. and was smaller than the right. No tumor nodule was detected grossly. Microscopically, the right gland showed diffuse moderate atrophy of seminiferous tubules, with thickening and hyalinization of basement membranes. The tubules were lined

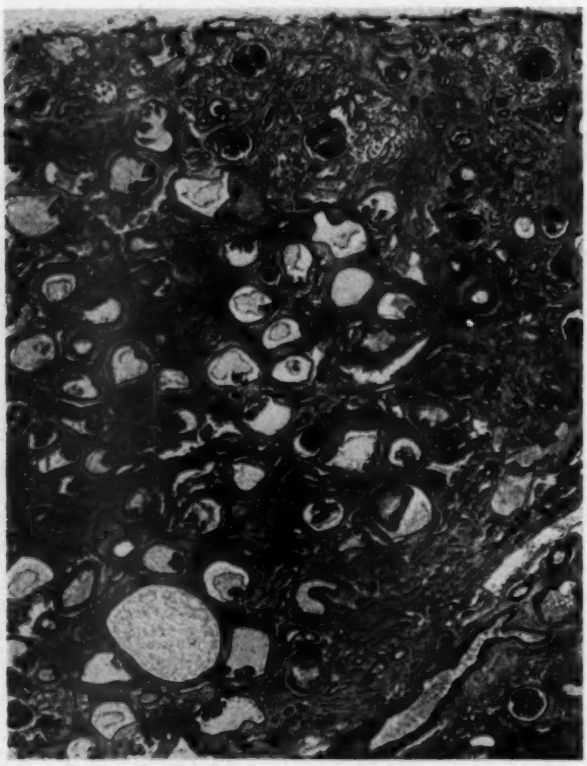

Fig. 7.—Typical low-power appearance of kidney. The cystic structures are glomerular remnants (× 48).

by Sertoli cells with a moderate number of spermatogenic cells, some in mitosis. No spermatozoa or spermatids were seen. Interstitial cells were seen in large and small clusters. The left testis presented a more noticeable degree of atrophy than the right. The tubules were smaller; the basement membranes were thicker, and the stroma was more compact and fibroblastic. Again, interstitial cells were prominent but were diffusely scattered, rather than in clusters. Four contiguous microscopic lobules of tumor tissue were found lying in the left testis beneath the tunica albuginea and with no apparent relation to the rete or epididymis. The lobules consisted of folliculoid arrangements of small uniform cells bounded by thin fibrous capsules

(Fig. 8). In places small amounts of amorphous material intervened between the tumor cells and the capsule. The cells were compactly arranged in anastomotic strands to outline numerous round spaces containing inspissated, and sometimes laminated, material. The nuclei were fairly uniform in size and shape, and no mitotic cells were found. Around the spaces with their concretions, the nuclei frequently were found lying next to the space with little or no cytoplasm intervening. The cells were consistent in all respects with granulosa cells, and the spaces were similar to Call-Exner bodies.

Gynecomastia was present grossly, with characteristic stromal increase and ductal dilatation microscopically.

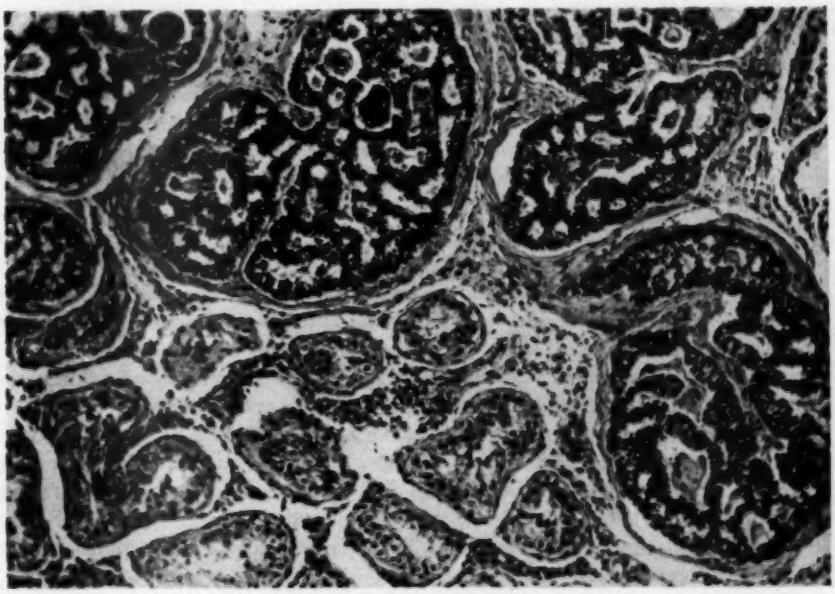

Fig. 8.—Granulosa cell tumor of testis (× 120).

The four parathyroid glands were equally enlarged, measuring 1.5 by 1 by 0.7 cm. and having a total weight of 1.5 gm. Microscopically their structure was in no way remarkable, with the bulk of the tissue consisting of principal cells.

Diffuse suppurative parotitis was present. The thymus was hyperinvoluted. The adrenals were enlarged and lipid-laden. The thyroid was slightly increased in size but was otherwise unremarkable. The pituitary gland weighed 0.5 gm. and presented a normal microscopic structure, except for scattered enlarged basophilic cells with vacuolization.

COMMENT

This unusual case represents a bewildering array of pathological findings; its diversity provides material of interest in many fields. Clinically, the generalized osteitis fibrosa, the leontiasis ossea, and the slipped epiphyses dominated the picture, and they will be discussed first. The mechanism by which extensive resorption of

bone and production of osteitis fibrosa occurs is not known.[1] Acidosis in the present case was not profound, and, in general, the progression of the bone changes did not parallel the severity of the acidosis. The concentration of calcium and phosphorus in the serum, together or singly, did not show any consistent relationship to the rate of bone depletion either. While hyperparathyroidism undoubtedly existed, as shown by a positive Hamilton-Schwartz test and diffuse parathyroid hyperplasia, this probably was secondary. A review [2] of the possible primary factors which may set off the sequence of chemical changes in the blood leading to generalized resorption of bone mentions the following: increased parathyroid hormone in the serum, hypocalcemia, hyperphosphatemia, lowered serum pH, and loss of fixed base. By the evidence in this case, none of these theories concerning the primary mechanism is to be preferred over the others. The serum content of many of these substances fluctuated more or less from day to day, and therefore the depletion may have been a discontinuous process not demonstrable as an over-all trend related to any particular chemical determination in serum. Passing mention may be made of one other theory, that of diencephalic dysfunction, merely to state that no gross or microscopic lesions were demonstrable in that area.

The leontiasis ossea represented overgrowth of one area of the skeleton in which the histological features resembled the generalized osteitis fibrosa. Why changes of this type should have occurred in the cranial bones was puzzling, and the date of onset of the cranial change could not be associated with any significant alteration of the kidney function or the degree of acidosis. The piling up of large amounts of new bone not situated so as to give mechanical support in areas of stress was an anomalous occurrence in a patient whose bones were all being slowly depleted. One would expect no such extravagant waste by the body's economy if our conception that bone is resorbed in order to maintain a serum-calcium level and to neutralize acid wastes is correct. Other unknown factors must have been present since all other reported instances of leontiasis ossea either have been isolated disturbances or have been associated with generalized disease, such as Paget's disease or polyostotic fibrous dysplasia without notable changes in serum electrolytes.[3]

The slipped femoral epiphyses were similar mechanically to the more common slipped epiphyses of idiopathic type. Slipping of the humeral epiphyses, while in an unusual location, also represented the expected mechanical derangement due to softening of the metaphyses. Albright and Reifenstein [4] mentioned two cases of slipped femoral epiphyses due to renal disease but did not describe the pathology. From the description given previously, it seems evident that in our patient the slipping occurred just distal to the epiphyseal cartilage. The fragmentation of the epiphyseal cartilage may be interpreted as part of the normal process of fusion of epiphysis to shaft. Comparison was made of the pathology of slipped epiphysis in this patient with that of a girl 13 years old who died of renal disease with only minor osteitis fibrosa. In the latter case, which is unpublished, there was also severe bilat-

1. Follis, R. H., Jr.: Renal Rickets and Osteitis Fibrosa in Children and Adolescents, Bull. Johns Hopkins Hosp. **87**:593-615, 1950.

2. Ullmann, T. D., and Schorr, S.: Renal Dwarfism with Hyperparathyroidism in a Case of Congenital Familial Malformation of the Kidneys, Ann. Int. Med. **29**:715-730, 1948.

3. Windholz, F., and Cutting, W. C.: Leontiasis Ossea, Stanford M. Bull. **3**:69-81, 1945.

4. Albright, F., and Reifenstein, E. C., Jr.: The Parathyroid Glands and Metabolic Bone Disease: Selected Studies, Baltimore, Williams & Wilkins Company, 1948.

eral slipping of one year's duration which occurred just distal to the epiphyseal cartilage plate in an area of intense bony resorption and fibrosis. The epiphyseal plate was intact and showed only those changes characteristic of growth arrest.

We believe that the megaloureters may be explained partly or completely by a deficiency in urinary tract innervation since obstructive lesions were absent.[5] Chronic pyelonephritis was superimposed on kidneys that, judging by the extremely small renal arteries, may well have been hypoplastic from birth. The renal insufficiency demonstrated during seven years of observation probably existed for a long period prior to the onset of symptoms, in view of the growth record and the fact that polyuria had been present from infancy. The growth record had been kept from the time the patient was 6 years old, and it followed the curve of the third percentile until growth ceased at the age of 17. The renal disease may not have been the only cause for the retarded growth, since in aortic coarctation retardation of growth also occurs.

The medical aspects of this patient's renal disease were unusual also, and the electrolyte distribution patterns in the Table will bear scrutiny. Records of the serum nonprotein nitrogen showed no consistent rise until the last year of the patient's life, although during any one period of multiple tests there was rather wide fluctuation. In general, the serum phosphorus followed the same pattern but with little fluctuation. The levels of serum calcium showed a prolonged response to the treatment of the acidosis without coordinate change in the phorphorus level. Changes in the serum sodium and potassium were not extensive, and the slight depression of chloride was not impressive. Therefore, the main feature of the electrolyte pattern in the serum was the maintenance of compensation for so long a period despite renal insufficiency of severe degree.

The presence of a testicular granulosa cell tumor of microscopic size is a startling finding, hitherto unreported to our knowledge. From an embryological standpoint, there is no reason why this should not occur. Testes and ovaries arise from the same anlage, and anomalous differentiation is not excessively rare (for example, ovotestis). There is no clear-cut evidence of estrogenic function of the tumor, but the following observations suggest that this may have been the case. The right testis presented a degree of atrophy fairly consistent with the patient's long-standing illness, but the testis containing the tumor was excessively atrophied. Bilateral gynecomastia was present, and the pituitary gland contained large vacuolated basophiles resembling castration cells.

Two other features of this case are worthy of comment. The pituitary, thyroid, and adrenal glands were larger than normal, and, although pituitary stimulation may be invoked to explain the increased size of the latter two, essentially the mechanism is unknown. The calcification of peripheral arteries developing in the last year of life is puzzling also. Despite the difference in pressure distal and proximal to the coarctation, the process was the same as observed roentgenologically in the upper and lower extremities.

This case is reported in detail because several features of it invite speculation on the inter-relations between the kidneys and endocrine secretions and suggest an experimental approach to their nature and effects on the metabolism of bone. One

5. Swenson, O.; MacMahon, H. E.; Jaques, W. E., and Campbell, J. S.: A New Concept of the Etiology of Megaloureters, New England J. Med. **246**:41-46, 1952.

may suspect that some of the bone changes are due not only to the effects of increased parathyroid activity and alteration of the serum electrolytes but also to the increased action of estrogens. This line of thought was entertained by Bremer [6] in relation to osteitis fibrosa. A second speculation is concerned with the relationship between prolonged renal insufficiency and the elaboration of a testicular tumor. It is possible that the reduced renal elimination of pituitary gonadotropic hormone, particularly during puberal change, may be significant etiologically, even as reduced inactivation of estrogens occurs in liver disease. Experimentally, renal insufficiency in weanling animals carried through the puberal period receiving the available estrogenic or pituitary preparations may clarify the pathologic physiology of this complex case.

SUMMARY

This is a case report of a 21-year-old man who presented the following principal pathological lesions: hypoplastic kidneys; chronic pyelonephrosis; leontiasis ossea; hyperparathyroidism, slipped epiphyses, and osteitis fibrosa secondary to renal disease; granulosa cell tumor of the testis; congenital megaloureter and hydronephrosis possibly secondary to ganglion cell deficiency, and aortic coarctation. A seven-year study of his clinical course and serum electrolyte pattern are summarized.

6. Bremer, J. L.: Osteitis Fibrosa Localisata: An Experimental Study, Arch. Path. **32**: 200-210, 1941.

COARCTATION OF CELIAC ARTERY

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MADISON, WIS.

A RARE anomaly involving the celiac artery has been noted recently during a necropsy, namely, a coarctation. The only reported case of this condition that we find is that described by Thane [1] in 1888 before the Anatomical Society of Great Britain and Ireland. In this specimen the celiac artery was completely obliterated at its origin from the aorta and replaced by a fibrous cord. The collateral circulation was established through the pancreaticoduodenal arteries. He offered no reason for this occlusion and could identify no disease process as the cause. There are numerous articles concerning aberrant vessels and the collateral circulation in the area of the celiac axis, but no satisfactory explanation has been described for a coarctation at this site.

REPORT OF A CASE

This lesion was observed during a necropsy on a 39-year-old white man who was addicted to alcohol and who had had a long history of bleeding from esophageal varices on the basis of an advanced Laennec's cirrhosis. During the last admission the esophageal bleeding was controlled by the insertion of a Patton tube and treated conservatively. In view of the serious nature of his disease and his inability to cooperate on a medical regimen, ligation of the left gastric, splenic, and hepatic arteries was contemplated. The prothrombin level prior to operation was 40%, and there was no response to therapy because of the extensive liver damage. The bleeding time was one and one-half minutes, and the clotting time (Lee-White) was eight and one-half minutes. Approximately three hours following the above surgical ligation the patient died with a massive abdominal hemorrhage (5,000 cc). Bleeding resulted from generalized oozing from the operative site, rather than from an uncontrolled bleeding point. The prothrombin level just prior to death was only 16%.

The liver weighed 2,750 gm. and presented a firm markedly nodular surface. On cut section the liver parenchyma was extensively replaced by fibrous connective tissue, and only occasional islets of regenerating liver were observed. Microscopically, scattered hemorrhagic islands of recognizable liver cords comprising about 20% of the section were found throughout the dense fibrous stroma. The biliary system presented foci of duct prolieration (Fig. 1). The spleen weighed 500 gm., with a hemorrhagic congested pulp. The distal 6 cm. of the esophagus contained beneath its mucosal surface dilated tortuous collapsed veins compatible with the anatomical diagnosis of varices.

The coarctation of the celiac artery extended for a distance of 1 cm. from its origin and then suddenly expanded into a fusiform-shaped vessel of normal proportion. The vessels arising from the celiac artery distal to the coarctation were the accessory left gastric, left gastric, splenic, and hepatic arteries, respectively (Fig. 2). The latter three were adequately ligated with black cotton sutures that served as a means of identification. The point of narrowing would admit the stylet of a 22 gauge needle.

Multiple sections taken through the constricted part, as well as distal and proximal to it, revealed only extensive arteriosclerosis, with a thickened intima and focal calcific and cholesterol deposition (Fig. 3). No histologic phantom could be

From the Department of Pathology, University of Wisconsin Medical School.

1. Thane, P. T.: Obliteration of Cœliac Axis, J. Anat. & Physiol. **22**:27, 1888.

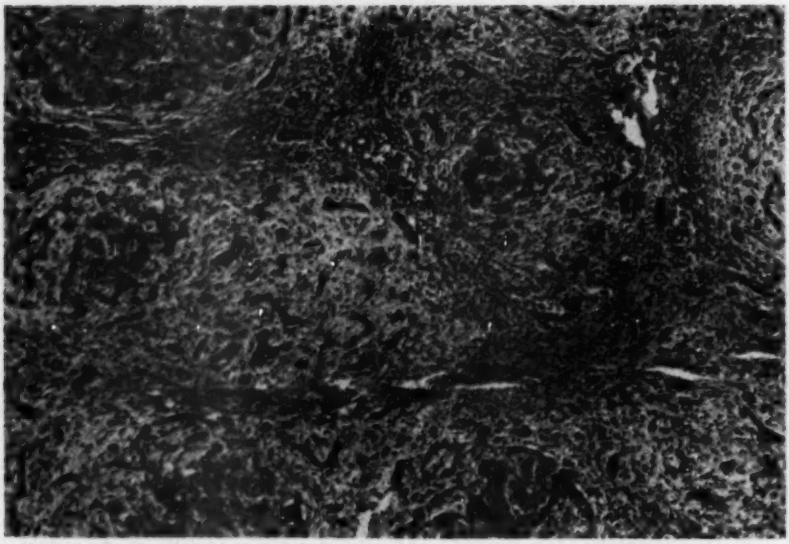

Fig. 1.—A section of liver showing the marked fibrosis and mononuclear cellular infiltrate surrounding the degenerating and regenerating islands of liver cords. Hematoxylin and eosin; × 90.

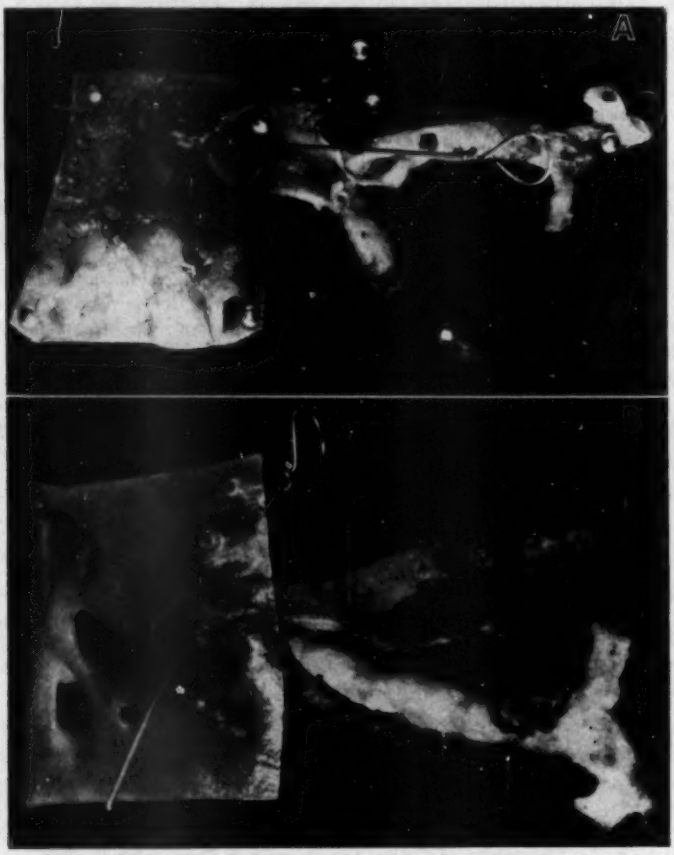

Fig. 2.—*A*, the celiac axis, showing the point of coarctation at its origin from the aorta. The stylet of a 22 gauge needle is shown inserted into the area of narrowing. The celiac artery distal to the coarctation is open and of normal dimension, with the hepatic and splenic arteries arising from it. *B*, the luminal aspect of the aorta, showing the narrowed celiac orifice and the style extending through it. Beneath is the orifice for the superior mesenteric artery and the right and left renal arteries.

seen which might give some lead as to the origin of this narrowing. It is our opinion that these arteriosclerotic changes are secondary to the preexisting embryonic coarctation and play no etiologic role in its development.

The origin and response of the vascular lesion raised certain problems. Julius Tandler,[2] in his treatise "Über die Varietäten der Arteria coeliaca und deren Entwickelung," describes the origin of the celiac axis and superior mesenteric artery as arising from four embryonic branches which originally unite the omphalomesenteric artery to the aorta. There is a later degeneration and subdivision of this system to form the superior mesenteric artery and the celiac axis. Many variations of this process of division are described, leading to different anatomical alterations which he diagrams and discusses. The concept of the origin of the celiac axis from this

Fig. 3.—The wall of the celiac artery at a point midway between the area of coarctation. This shows the intimal thickening, loss and fragmentation of the elastic fibers, and the focal deposition of calcium and cholesterol. Hematoxylin and eosin; × 90.

work offers the possibility of partial occlusion at the time the embryonic framework develops by this process into the permanent vascular elements of this area.

Recently Michels [3] stated that dextrorotation of the gut takes place about the superior mesenteric artery and that this artery rotates 180 degrees during this process. He describes visible spiral grooves which impinge on its lumen as proof of this previous rotation. He noted that the celiac artery was narrowed in a few instances at its site of origin from the aorta, one instance simulating an exact reduplication of the lesion described here. He could find no lesion to explain this narrowing and considered the process to be associated with embryonic development, more especially a by-product of gut rotation.

2. Tandler, J.: Über die Varietäten der Arteria coeliaca und deren Entwickelung, Anatomische Hefte. **25**:473, 1904.

3. Michels, N. A.: Personal communication to the author.

What contribution this early coarctation may have played in the genesis and development of the cirrhosis can only be speculative because of its alteration of the regional blood supply to the liver. Michels [3] has determined 26 possible collateral arterial pathways to the liver, one example of which would permit blood from the superior mesenteric artery to reach branches of the celiac, thereby reestablishing normal blood flow.

SUMMARY

A coarctation of the celiac axis at its origin from the aorta is described in a case of far-advanced Laennec's cirrhosis. The most acceptable modern concept as to its derivation rejects any pathological entity and proposes that it is due to early fetal gut rotation affecting this portion of the aorta and its branches. Gross spiral grooves have been described impinging on the lumen of the superior mesenteric artery, and a similar process could be responsible for this marked narrowing of the celiac axis.

A UTERUS IN A MAN

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WINSTON-SALEM, N. C.

THIS paper discusses the occurrence of a unicornuate uterus in a man. The structure was found incidentally during a gastrectomy and was removed surgically at that time. Relevant embryologic relationships are presented.

A 57-year-old divorced accountant, the father of one child, was admitted to the North Carolina Baptist Hospital because of bleeding from a duodenal ulcer. The ulcer had been present for at least 10 years, and he previously had had a perforation and several episodes of bleeding. The principal physical findings were an old abdominal scar and a reducible right inguinal hernia. The external genitalia were normal, and the testes were descended. Since medical treatment had failed to control his bleeding, a subtotal gastrectomy was performed. Routine abdominal exploration at the time of operation disclosed a small firm mass in the rectovesical space, slightly to the right of the midline. The right kidney was absent, and the surgeon felt that the small pelvic mass represented a hypoplastic kidney. There were several fibrous attachments, one extending toward the inguinal canal and another toward the bladder. The latter was thought to be a ureter. The mass and its fibrous attachments were removed. The patient's postoperative course was marred by a period of fever, which was attributed to leakage from the duodenal stump. No further investigations relative to the genitourinary tract were carried out postoperatively since they were not concerned with the patient's welfare.

The specimen received in the pathologic laboratory is pictured in Figure 1. It was an ovoid mass of resilient brown tissue, 3.5 by 2.5 cm., with a prominently vascular capsule. Three equatorial projections, partially crushed, were present. Two were fibrous. The third was 0.5 cm. long and 0.3 cm. in diameter; its lumen was visible but was not probed. On section the specimen was composed of slightly bulging white whorled tissue, with a central compressed cleft continuing into the tubular projection previously mentioned. The whorling and bulging of the wall were strongly suggestive of myometrium. Microscopically the wall of the structure consisted of interlacing bundles of smooth muscle (Fig. 2). The central cavity was lined by columnar epithelium, with underlying tubular glands in a sparsely nucleated stroma (Fig. 3). The tubular portion of the specimen was partially lined by papillary fronds of connective tissue with surface epithelium similar to the main cavity (Fig. 4), and the remainder was lined by flattened unidentifiable epithelium. The specimen was interpreted as a hypoplastic uterus and Fallopian tube, and its situation in the body indicated that it was unicornuate. The "Fallopian tube" may also represent the endocervix.

Those qualified observers who have examined the specimen have all thought it most closely resembled a uterus. One or two have been unwilling to call it by that name since the epithelium is not completely typical for the uterus. However,

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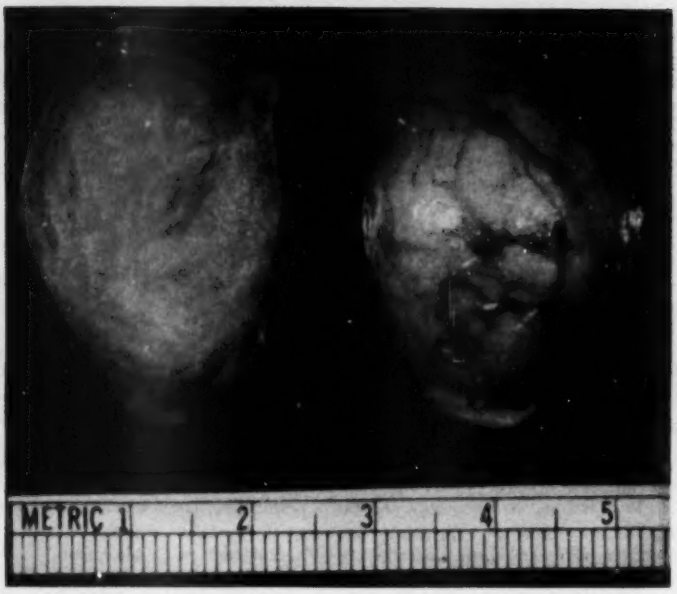

Fig. 1.—External and sectioned surfaces of the mass. The epithelial-lined cleft extends from the projection of the upper right of the sectioned surface diagonally toward its center; × 2.

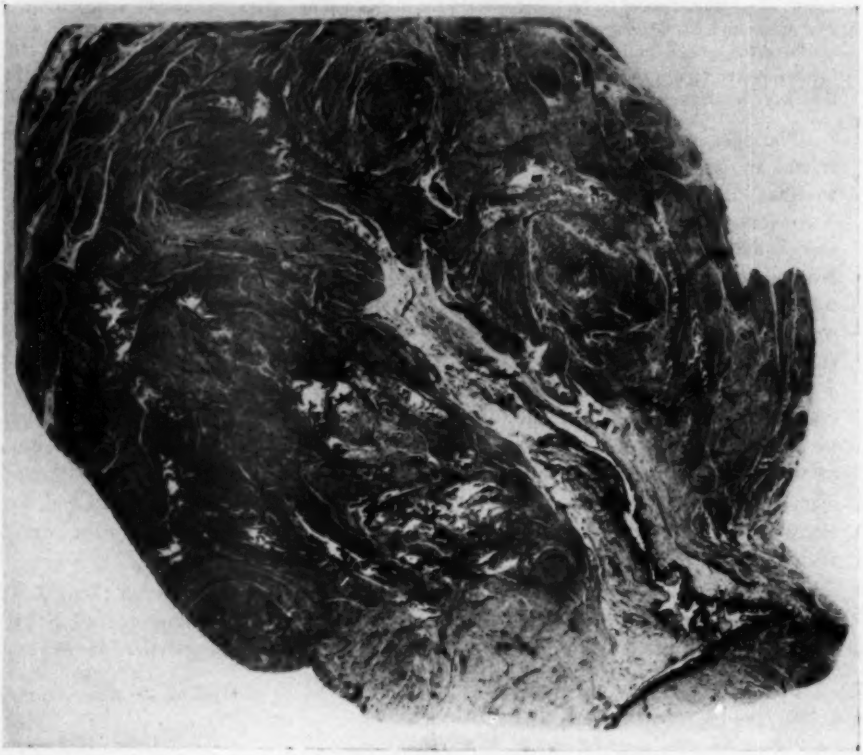

Fig. 2.—The character of the smooth muscle wall and the epithelial-lined central cavity is shown; × 4.

certain modifying factors must be taken into account in the present case. First, the epithelium had been exposed for 57 years to the influence of a male hormonal pattern, and, second, it had existed for that many years without ever maturing in the fashion of normal uterine epithelium. The epithelium lining this structure is as well or better preserved than the epithelium lining such structures as the prostatic utricle or appendix testis, the closest, though admittedly inadequate, parallels which can be offered. It closely resembles the epithelium of the uterus and Fallopian tube in female children (Figure 5).

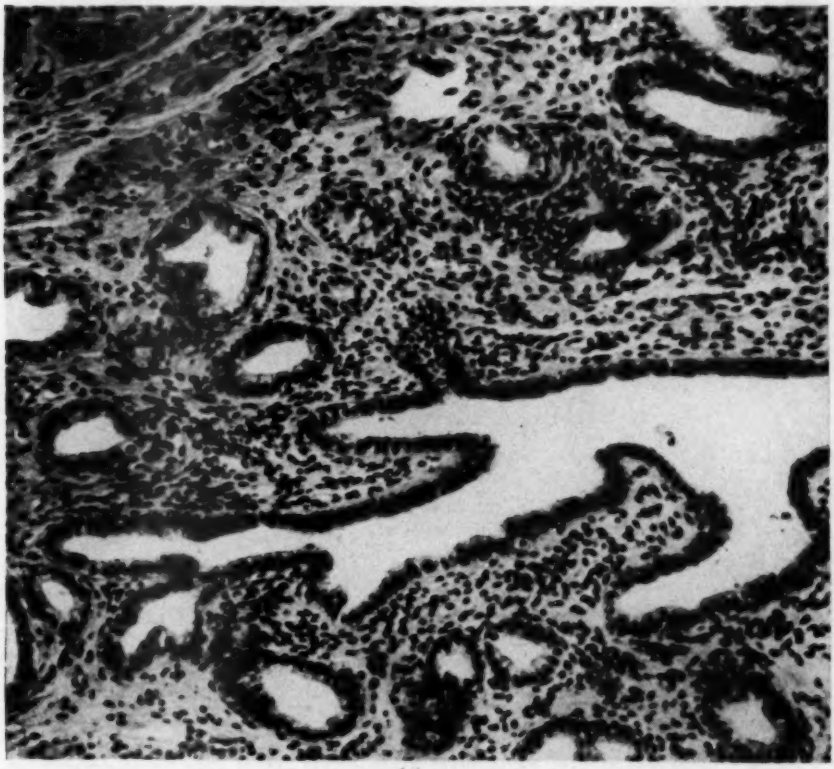

Fig. 3.—High-power view of the epithelium lining the central cavity, with tubular glands and sparsely nucleated stroma; × 198.

A very brief review of the development of relevant structures[1] may be of aid in assessing the situation in this patient. The human Müllerian duct arises on the surface of the Wolffian body in the 10 to 11 mm. embryo, adjacent to the meso-

1. Patten, B. M.: Human Embryology, Philadelphia, The Blakiston Company, 1946, pp. 549-607. Faulconer, R. J.: Observations on the Origin of the Müllerian Groove in Human Embryos, Contrib. Embryol. Carnegie Inst. **34**:159-164, 1951. Gruenwald, P.: The Relation of the Growing Müllerian Duct to the Wolffian Duct and Its Importance for the Genesis of Malformations, Anat. Rec. **81**:1-19, 1941.

nephric duct. The metanephric diverticulum, which participates in the development of the human kidney, appears slightly earlier, i. e., in the 5 to 6 mm. embryo. In the normal course of events the mesonephric ducts in the male (Fig. 6) form the duct system of the testis, while the Müllerian duct atrophies. The usual Müllerian remnants are the prostatic utricle and the appendix testis. The metanephric duct grows into the metanephric blastema and initiates formation of the permanent kidney. In the female the Müllerian ducts form the uterus, uterine tubes,

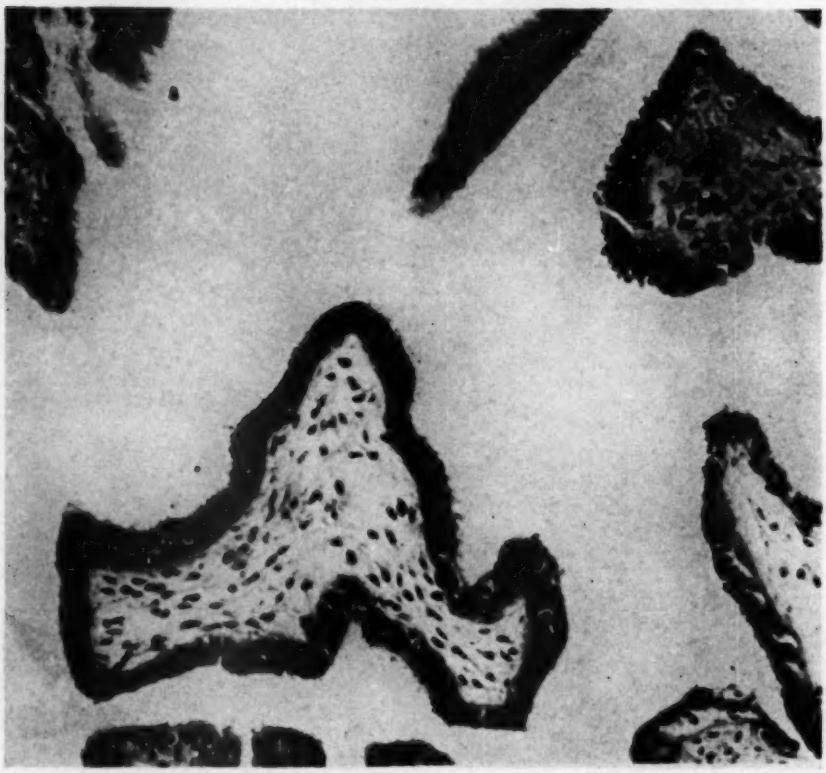

Fig. 4.—High-power view of the papillary fronds of the tubular structure attached to the main mass. Note the ciliated cells and the "peg" cells; × 162.

and vagina, while the mesonephric ducts atrophy; their remnants are seen as the epoophoron and the canals of Gartner. In its growth laterally the metanephric duct crosses the Müllerian duct.

In the present patient (Fig. 7) it seems that the mesonephric duct system formed the excretory ducts of the right testicle, as usual. The metanephric duct, however, apparently never developed and, in any case, failed to reach the metanephric blastema and form a kidney. The right Müllerian duct did not regress but formed the structure removed at operation . At this time, we do not know if any other structures of Müllerian origin are present elsewhere in this patient. The rela-

tionship between persistence of the right Müllerian duct and the failure of the kidney to develop is unknown, but we do know that the coexistence of renal and genital anomalies is frequent and is often associated with indirect inguinal hernia.[2]

It is quite difficult to determine whether any cases of this type have been previously reported, since minute variations in broadly similar cases may lead to a variety of diagnoses. I was not able to find an exact parallel for the present case in the literature, however. When a structure so definitely uterine is present in the male, there are usually other anomalies constituting pseudohermaphrodism, and hypospadias and undescended testicles are almost always present. Cysts arising from

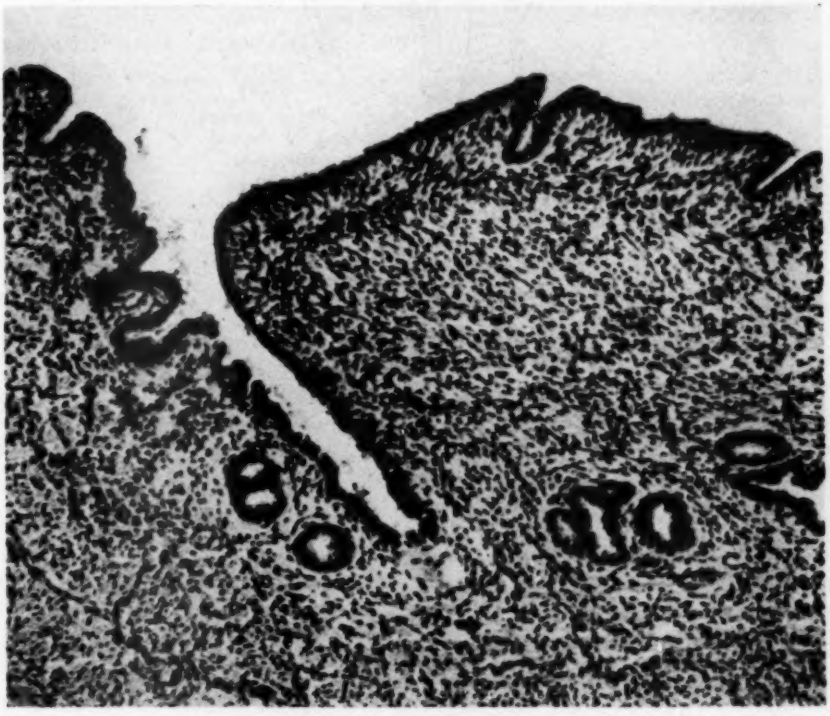

Fig. 5.—Uterine wall of an 18-month-old girl for comparison with Figure 3; × 198.

Müllerian duct remnants rarely cause clinical difficulties leading to operation, although 15 have been reported [3] in roughly the same area as the structure found in

2. Shumacker, H. B., Jr.: Congenital Anomalies of the Genitalia Associated with Unilateral Renal Agenesis with Particular Reference to True Unicornuate Uterus: Report of Cases and Review of the Literature, Arch. Surg. **37**:586-602, 1938. Drummond, D. H., and Palmer, H. D.: Unilateral Renal Agenesis with Associated Genital Anomalies, J. Urol. **42**:317-320, 1939. McCahey, J. F.: A New Conception of Hermaphroditism, Surg., Gynec., & Obst. **67**:646-654, 1938. McKenna, C. M., and Kiefer, J. H.: Unusual Anomaly of the Urogenital Tract Associated with Hypospadias, Urol. & Cutan. Rev. **47**:14-20, 1943. Middleton, R. P.: A Case of Pyometra in a Male Pseudohermaphrodite, New England J. Med. **204**:902-904, 1931.

3. Landes, R. R., and Ransom, C. L.: Müllerian Duct Cysts, J. Urol. **61**:1089-1093, 1949.

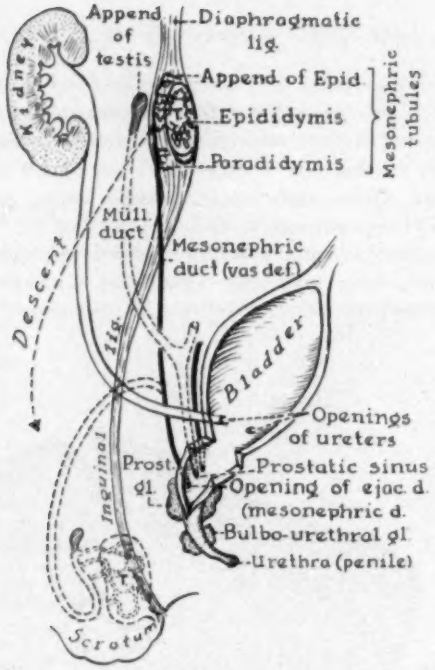

Fig. 6.—Diagrammatic outline of the development of the male urogenital system (modified from Patten).

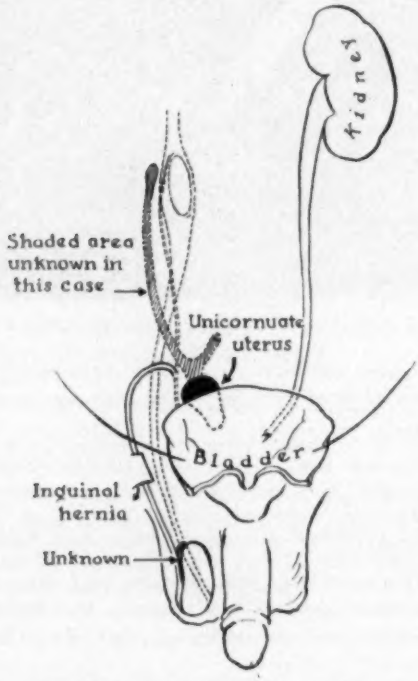

Fig. 7.—Diagrammatic outline of the probable variations in the present patient. The fate of the other portions of the Müllerian duct is unknown.

the present patient. In this same region cystic Müllerian remnants are found incidentally in about 1% of autopsies.[3] The present structure, however, probably represents a more fundamental embryonic disturbance than the presence of cysts such as those mentioned above since the ipsilateral kidney was absent and there was an indirect inguinal hernia. The case represents another member of the large family of combined urogenital anomalies, again emphasizing the interrelationship of the two systems.

SUMMARY

A structure resembling and probably representing a hypoplastic unicornuate uterus was found incidentally during an operation on a man. The patient also had a right indirect inguinal hernia, and his right kidney was absent. Pertinent embryologic relationships are discussed briefly.

Drs. Howard Starling, Bradley Patten, Peter Gruenwald, and Robert Faulconer aided in the preparation of this report. Mr. Hooker Goodwin of the Department of Medical Illustration, Bowman Gray School of Medicine of Wake Forest College, prepared the diagrams.

General Reviews

PATHOLOGY OF EPIDEMIC TYPHUS

Report of Fatal Cases Studied by United States of America Typhus Commission in Cairo, Egypt, During 1943-1945

Prepared by the

COMMITTEE ON PATHOLOGY

DIVISION OF MEDICAL SCIENCES, NATIONAL RESEARCH COUNCIL

With Collaboration of the

ARMED FORCES INSTITUTE OF PATHOLOGY

CENTRAL NERVOUS SYSTEM

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(Concluded from Page 435)

RÉSUMÉ

TYPHUS produces changes in the brain and spinal cord almost identical with those in the skin and subcutaneous tissue, including the focal lesions of microscopic size usually referred to as nodules, perivascular accumulations of mononuclear cells, and infiltrations of mononuclear cells in the meninges. Small hemorrhages in the substance of the brain or spinal cord and in the meninges and degeneration of ganglion cells are common but should probably be considered incidental. The focal lesions or nodules take origin in blood vessels, chiefly capillaries and precapillaries, and are a proliferative reaction to injury of the endothelial cells by the rickettsiae. The lesions are composed in large part of two cellular elements, those derived from the vascular endothelium and those from the neuroglia. The cellular composition of the nodules is not static, and it varies with the severity of the initial reaction and the length of survival (Wolbach, 1948). At an early period following the initial vasculitis, macrophages of mesenchymal origin predominate, followed soon by migration of ameboid neuroglia cells. Neutrophilic granulocytes appear for a period and may predominate if there has been necrosis of tissue. Satellitosis, degeneration, and necrobiosis of ganglion cells occur. These changes may persist for as long as eight weeks after recovery from typhus, and in late periods astrocytes are found in the nodules. The final stage is a minute neuroglial cicatrix. A variant of the typhus nodule called a microinfarct, which is of unknown nature, has been described and will be discussed in detail later (Lillie, 1941). The perivascular and meningeal accumulations are principally mononuclear cells—macrophages, which are thought to be monocytes, endothelial leucocytes, lymphocytes, and plasma cells. All portions of the brain and spinal cord may be attacked, but the inferior olivary nuclei are outstandingly and selectively involved. Other areas may be arranged according to the relative intensities of lesions into a series of four poorly separated groups. Group 1 includes the cerebral cortex, nuclei of the pallidonigral system, major

regions of the nuclei of the cranial nerves, gray matter of the spinal cord, and molecular cortex of the cerebellum; Group 2 includes the nuclei of the neostriatum, the inflow and outflow nuclei of the cerebellum, two primitive cortical areas associated with olfaction, and the pons; Group 3 includes regions of intermixture of white and gray substance, the nuclei of the diencephalon and the claustrum and amygdala; Group 4 is composed of the regions of white matter. It seems likely that the differences between these groups are directly related to the number of nerve cells and blood vessels in the area and inversely to the amount of myelin. Other factors, such as the concentrations of axons, dendrites, and synapses, may also be operative.

The lesions in the central nervous system have been regarded as the cause of some of the nervous manifestations of epidemic typhus because of the demonstration of direct involvement of ganglion cells in the cerebral and cerebellar cortices and in many of the nuclei. Common symptoms thought to indicate cerebral lesions include headache, apathy, delirium, stupor, and muscular twitchings. More severe manifestations, such as coma and incontinence of feces and urine, also occur. In severe cases the limbs may become spastic, the reflexes hyperactive, and the Babinski test positive. Catatonia affecting especially the arms, continuous picking of the bed clothes, and athetoid movements of the limbs may develop. Since many of these patients also show azotemia, some of these manifestations may be due to this rather than to a direct action of the rickettsiae. Recovery, when it occurs, is usually complete, and there are no residuals to indicate permanent injury of the central nervous system. Deafness and tinnitis aurium sometimes occur and may persist for several weeks during convalescence.

CAIRO CASES

Sections of the brain were available in 29 cases and of the spinal cord in 26. The brain was involved in 26 cases and the spinal cord in 17, and the lesions were the same in both. The principal lesions were (*a*) typical typhus nodules, (*b*) accumulations of mononuclear cells in the Virchow-Robin perivascular spaces, (*c*) diffuse and perivascular infiltrations of mononuclear cells in the meninges, and (*d*) so-called microinfarcts. The characteristic nodule was compact or loose and composed of round or elongated pyknotic or faintly chromatic nuclei in a ground substance which at times stained more intensely with eosin than did the surrounding tissue. In some instances the cytoplasm of the cells was not observed, but in other instances it formed a dense or light, round or fusiform mass. In most nodules there were small and large mononuclear cells and in an occasional nodule a few polymorphonuclear leucocytes.

Many nodules were arranged either eccentrically or concentrically about a small blood vessel which might show swelling and proliferation of endothelium, necrosis of the wall, or rarely a thrombus. In some nodules in which the relation to a vessel was not apparent in ordinary sections, reticulum stains with silver carbonate revealed tiny rings or ovals of reticulin in the center, identical with the appearance of capillaries. In a few nodules a vascular relation could not be proved, perhaps because of the level of the section.

Foci of necrosis similar to the lesions found in Rocky Mountain spotted fever and usually called microinfarcts (Lillie, 1941) were present in the white substance and showed large round or oval eosinophilic masses, clear vacuoles, and in one case (duration 17 days) glial proliferation. Necrotic arterioles occluded by a thrombus

could not be demonstrated in direct relation to the foci of necrosis, and in this respect the lesions differ from those in Rocky Mountain spotted fever. Thirty individual lesions were observed in 10 patients (Table 4).

Occasional examples of chromatolysis, neuronophagia, and satellitosis were observed. Within the brain the perivascular spaces contained lymphocytes, large mononuclear cells, and plasma cells. Swelling and proliferation of vascular endothelium were observed and at times necrosis of the wall and occlusion of the lumen by a hyaline thrombus or one containing fragmented nuclei. Occasional capillaries and arterioles showed leucostasis. Rarely hemorrhage into the sheath of a vessel or the perivascular space was present.

TABLE 4.—*Distribution of "Microinfarcts" in Brain and Spinal Cord*

Case No.	Duration of Typhus, Days	No. of Lesions	Location of Lesions
4	7	1	Tegmentum pontis
33	12	2	Brain
12	13	1	Spinal cord
17	14	1	Medulla at decussation, lateral column
13	15	1	Lateral column spinal cord
20	15	2	Tegmentum, midbrain and at decussation of brachia conjunctiva
30	16	13	4 anterior lateral and dorsal columns of spinal cord; 5 pons; 2 tegmentum (1 brachia, 1 pontile); 2 tegmentum midbrain
49	16	3	2 levels of spinal cord
22	17	6	1 corona radialis, cerebral cortex; 2 medulla at decussation; 3 spinal cord, anterior and lateral columns
14	32	2	Cerebral peduncle; medulla at decussation

TABLE 5.—*Influence of Duration of Typhus on Intensity of Reaction in Brain*

Death, Day	Grade of Involvement*				
	None	Slight	Moderate	Marked	Total
6th to 14th	65	60	39	12	176
	0.369	0.341	0.222	0.068	1.000
15th to 32d	28	31	29	9	97
	0.289	0.320	0.299	0.093	1.000
Total	93	91	68	21	273
	0.304	0.333	0.249	0.079	1.000

* Grade of involvement (lower figures) estimated on the basis of the number of sections examined (upper figures).

The intensity of infiltration of the meninges varied from case to case and area to area. There was no close correlation of the intensity of the intracerebral and meningeal lesions. Lymphocytes, large mononuclear cells, and plasma cells occurred diffusely and in perivascular position in the leptomeninges.

The choroid plexus was examined in one instance. There was slight lymphocytic infiltration both at the base and in the villi.

EFFECT OF DURATION OF DISEASE ON INTENSITY OF REACTION

In order to study the effect of the duration of typhus on the degree of involvement of the brain, the material was divided arbitrarily into cases fatal on or before the 14th day and those fatal on the 15th day or later, and the severity of the involve-

based on the total number of blocks available for examination in each of the two groups. It appears from this analysis that the percentage of areas showing no lesions was lower in the 15 to 32-day group than in the 6 to 14-day group and that there had also been an increase in the number of slight and moderate reactions in the first group.

DISTRIBUTION OF LESIONS OF EPIDEMIC TYPHUS IN CENTRAL NERVOUS SYSTEM

Selective distribution of lesions in the central nervous system is observed in several viral and rickettsial diseases. A reliable specificity of such selective distribution is most commonly acknowledged in the viral diseases, particularly poliomyelitis. It is not certain that rickettsial diseases exhibit diagnostically significant specificity in the distribution of their lesions. The material of the present study, therefore, presents an opportunity to evaluate that possibility in relation to epidemic typhus.

The lesions of epidemic typhus in the brain and spinal cord are essentially infiltrations of mononuclear cells about blood vessels. A division between typhus nodules, on the one hand, and perivascular infiltrates, on the other, is complicated by the presence of transitional forms. It seems apparent that such variation is due more to the size of the blood vessel involved than to an inherent difference in the lesion. It was therefore decided that in this study no attempt would be made to compile separate data on such divisions of the essential lesion. The meningeal infiltrations had no bearing on the information sought, and the microinfarcts described by Lillie in Rocky Mountain spotted fever (1941) and observed in this material were too sparse to give a useful pattern of distribution.

In order that a quantitative estimate of the distribution of the lesions might be made, it was necessary to exercise some selection of the material. As nearly as possible each case should contain sections from comparable areas in approximately comparable amounts, and it was desirable that cases with various degrees of total intensity of lesions be included in order to detect selective distribution that might be apparent only in lightly affected patients. The material from the total series was therefore reduced to that from 11 cases so that these requirements might be met.

Method.—Celloidin embedded blocks were sectioned at 20 μ and stained with cresyl violet and by Weil's method. A section 6 μ thick and stained with hematoxylin and eosin was also prepared. The sections stained by Weil's method were enlarged by optical projection and printed directly on photographic paper to give reproductions of each section at a magnification of 10 diameters. The myelin preparations were used in this step to facilitate identification and more exact measurement of the various areas. The areas of various regions on these photographs were measured with a planimeter, and the volume of tissue represented was calculated. The lesions in these regions were then identified in the sections and the results expressed as lesions per volume in cubic millimeters of embedded tissue, as tabulated in Table 6. By combination of the values for each region from the various cases and reduction of the expressions to lesion per cubic millimeter, Table 7 was prepared. In this table the various regions were arranged in descending order of the intensity of which lesions occurred. An expression of the relative frequencies of lesions in each region was obtained by calculating the ratio between the incidence of lesions in that region as compared to the incidence in cerebral white matter.

Table 8 was prepared to show the range of over-all intensity of lesions in the various cases. It represents simply the total number of lesions in each case divided by the total number of cubic millimeters of embedded tissue measured.

TABLE 6.—*Distribution of Lesions in Epidemic Typhus**

Region	Case No. 33	37	17	27	12	11	13	9	28	6	4	Totals
Cerebrum Cortex	10	4	8	0	23	25	30	15	36	75	80	306
	21.28	5.47	10.39	8.15	6.89	8.90	6.51	3.63	4.60	8.82	5.81	90.45
Putamen	3	..	2	0	6	24	10	13	3	0	9	70
	3.37	....	3.97	1.79	2.04	6.48	3.03	2.01	1.80	0.27	0.72	25.47
Caudate	0	..	0	0	2	20	6	2	3	0	2	35
	0.30	....	1.48	1.26	0.67	2.22	5.10	0.76	0.32	0.09	0.21	12.41
Claustrum	0	..	0	0	0	2	0	..	0	..	1	3
	1.00	....	0.62	0.42	0.43	0.48	0.65	....	0.14	....	0.12	3.86
Globus pallidus	0	0	0	0	8	0	0	0	1	..	..	9
	2.03	0.77	1.53	1.11	2.26	5.98	0.92	1.13	1.27	....	....	17.00
Amygdala	0	1	3	..	..	2	..	..	..	..	..	6
	0.79	1.33	1.65	....	....	2.41	....	....	....	....	....	6.18
Hippocampus	1	..	0	0	..	..	8	..	..	..	..	9
	0.54	....	0.76	1.28	....	....	0.82	....	....	....	....	3.40
Paraolfactory cortex	..	..	..	..	..	12	1	9	..	..	..	22
	....	....	....	....	....	4.68	0.45	2.85	....	....	....	7.98
Lateral geniculate	..	..	..	..	..	..	..	..	..	..	2	2
	....	....	....	....	....	....	....	....	....	....	0.22	0.22
Thalamus	1	0	0	2	3	..	2	..	9	20	20	57
	7.39	0.42	1.06	4.43	2.82	....	4.48	....	5.63	4.95	3.58	34.76
Hypothalamus	1	..	..	..	..	..	..	..	..	..	..	1
	1.03	....	....	....	....	....	....	....	....	....	....	1.03
Optic tract	..	0	..	..	..	..	..	..	..	..	..	0
	....	0.41	....	....	....	....	....	....	....	....	....	0.41
Internal capsule	0	0	..	0	0	..	..	..	..	..	..	0
	5.47	1.53	....	1.58	0.90	....	....	....	....	....	....	9.48
Cerebral white matter	0	0	2	0	1	3	4	0	7	16	14	47
	18.89	10.94	17.00	10.44	14.31	15.85	10.48	7.45	10.74	15.87	16.27	148.24
Cerebellum (dentate gray matter)	0	1	3	1	7	1	0	0	1	3	1	18
	0.09	0.50	0.95	0.92	0.70	0.66	0.50	0.71	0.52	0.26	0.19	6.60
Molecular cortex	1	2	6	16	10	16	20	10	29	33	22	165
	1.73	1.85	3.64	2.25	1.79	2.30	2.20	2.03	2.98	1.29	1.97	24.00
White matter; granularis	0	0	2	0	7	0	3	0	1	3	5	21
	4.46	6.42	7.86	5.78	4.01	5.41	4.53	5.56	6.66	3.55	5.40	59.04
Mesencephalon (periaqueductal gray matter)	4	2	..	..	..	..	1	..	1	..	..	8
	0.56	0.29	....	....	....	....	0.60	....	0.54	....	....	1.99
Reticularis	2	1	..	..	..	..	0	..	4	..	..	7
	1.88	0.85	....	....	....	....	1.52	....	1.84	....	....	6.09
Substantia nigra	1	1	..	..	..	..	7	..	..	1	4	14
	1.25	0.48	....	....	....	....	0.74	....	....	0.30	0.62	3.39
Pedunciles	0	0	..	..	..	..	0	..	..	..	..	0
	1.21	1.16	....	....	....	....	1.06	....	....	....	....	3.43
Brachia conjunctiva; red nucleus	0	0	..	..	..	..	0	..	2	..	..	2
	0.72	0.95	....	....	....	....	0.73	....	0.68	....	....	3.08
Pons (floor gray matter)	0	1	0	0	2	2	0	3	..	0	5	13
	0.10	0.15	0.08	0.32	0.26	0.33	0.09	0.49	....	0.10	0.25	2.17
Reticularis	0	1	0	1	10	3	2	7	3	2	26	55
	1.35	1.21	1.11	2.35	1.78	1.79	1.07	3.04	2.39	2.08	5.31	23.43
Basalis	1	5	2	7	7	19	13	26	6	11	61	159
	6.57	6.48	2.48	9.18	3.95	7.80	5.84	3.08	4.87	6.30	14.62	71.24
Brachia	0	..	0	..	1	6	0	10	..	5	3	25
	1.79	....	1.16	....	1.72	3.52	1.09	2.92	....	2.17	3.50	17.87
Medulla (floor gray matter)	1	1	0	1	4	6	0	1	2	2	9	27
	0.50	0.38	0.13	0.56	0.27	0.57	0.41	0.21	1.73	0.22	0.50	5.48
Reticularis	1	6	3	4	3	0	14	0	20	1	30	82
	1.41	2.87	0.60	2.30	0.72	1.60	1.20	0.43	3.46	0.86	2.52	18.06
White matter	2	0	0	2	7	3	4	2	5	11	11	47
	3.33	2.41	1.27	3.35	1.33	3.96	4.74	1.90	3.55	1.70	2.76	30.35
Olivary nuclei	1	1	5	8	6	26	21	1	21	7	19	122
	0.46	0.45	0.21	0.47	0.48	0.67	0.51	0.16	0.75	0.08	0.45	4.69
Pyramids	0	0	0	0	1	0	0	1	2	0	4	8
	1.12	1.54	0.35	0.84	0.47	0.87	1.27	0.79	1.19	0.41	0.45	8.80
Spinal cord (gray matter)	1	..	1	2	4	1	5	1	4	2	4	25
	1.38	....	0.35	0.87	1.08	0.31	0.82	0.46	1.08	0.34	0.23	6.92
White matter	1	..	0	0	3	0	1	0	2	0	0	7
	5.14	....	0.93	4.63	5.52	1.43	4.04	2.40	4.77	1.00	0.70	30.56
Totals	32	37	37	44	115	171	152	107	162	192	322	1,371
	97.74	48.86	59.58	64.26	54.34	77.90	66.30	42.07	61.60	50.06	66.40	669.71

* Upper figure indicates number of lesions; lower figure indicates cubic millimeters.

For practical purposes the lesions included in the tabulations were those that were readily identified with a magnification of 32 diameters. More detailed tallies were prepared from the slides of Case 13 by enumerating all lesions that could be discerned by magnification up to 400 diameters and by measuring the areas of tissue from tracings prepared by projection at 13.75 diameters magnification. The relative values of the different figures obtained by this method did not vary sufficiently from those of the simpler method to justify conducting the entire study in that manner. No attempts were made in these studies to correct for shrinkage of tissue. It was assumed the tissues from the various patients were handled in a reasonably similar manner. However, expression of the results is given in relative terms to allow for distortion by such artifacts.

TABLE 7.—*Distribution of Lesions in Epidemic Typhus by Totals of Areas Measured*

Region	Lesions per Cu. Mm.	Ratio to White Matter
Inferior olivary nuclei	26.0	87.2
Lateral geniculate	9.10	30.6
Cerebellar molecular cortex	6.87	23.1
Pontine floor gray matter	6.00	20.2
Globus pallidus	5.29	17.7
Medullary floor gray matter	4.98	16.5
Medullary reticularis	4.82	16.2
Mesencephalic substantia nigra	4.13	13.8
Mesencephalic periaqueductal gray matter	4.02	13.5
Spinal cord gray matter	3.61	12.1
Cerebral cortex	3.38	11.3
Caudate nucleus	2.84	9.47
Paraolfactory cortex	2.76	9.26
Dentate gray matter	2.73	9.16
Putamen	2.64	8.86
Hippocampus	2.64	8.86
Pontine reticularis	2.34	7.86
Pontine basalis	2.22	7.45
Thalamus	1.64	5.50
Medullary white matter	1.55	5.20
Pontine brachia	1.40	4.70
Mesencephalic reticularis	1.15	3.86
Amygdala	0.970	3.26
Hypothalamus	0.970	3.26
Medullary pyramids	0.909	3.04
Claustrum	0.778	2.61
Mesencephalic brachia; red nucleus	0.650	2.18
Cerebellar white and granular matter	0.352	1.18
Other cerebral white matter	0.317	1.06
Total cerebral white matter	0.298	1.00
Spinal cord white matter	0.229	0.768
Internal capsule	0.0	0.0
Optic tract	0.0	0.0
Mesencephalic peduncles	0.0	0.0

Results.—Table 8 shows the general intensity of lesions and the volumes of tissue measured in each case. The near coincidence of the average and mean values suggests this series is about equally composed of patients with lightly and heavily affected nervous tissues. By analysis of the figures in Table 6, no distinct differences are found between the distribution of lesions in lightly as contrasted to heavily affected patients.

The results of this study are summarized in Table 7. The various regions measured present a nearly continuous gradient in the incidence of typhus lesions. The inferior olivary nuclei contain three times as many lesions as any other region. After them the series can be divided approximately into quarters without too clear physiological or anatomical significance of the included members. The cerebral

cortex, nuclei of the pallidonigral system, major regions of the nuclei of the cranial nerves, gray matter of the spinal cord, and molecular cortex of the cerebellum are in the first group. In the second group the nuclei of the neostriatum, the inflow and outflow nuclei of the cerebellum, two primitive cortical areas associated with olfaction, and the pons contain between 7.45 and 9.47 times as many lesions as white matter. The third group down to the claustrum is composed essentially of regions of obvious intermixture of white and grey substance, with a preponderance of the former, plus the nuclei of the diencephalon and the claustrum and amygdala. The regions in the final group are almost purely white matter.

For most of the regions a fair sample is represented. It should be noted, however, that the values for the lateral geniculate body and hypothalamus are based on only one section each. The lateral geniculate body is in the most intensely involved case and the hypothalamus in the least. Also, the mesencephalon is only sparsely represented in the various cases, and the values for amygdala, hippocampus, and paraolfactory cortex are based on material from only a few cases.

TABLE 8.—*Distribution of Lesions in Epidemic Typhus*

Case No.	Lesions	Lesions per Cu. Mm.*
33	32 in $9{,}774 \times 10^{7}$ cu. μ, or 97.74 cu. mm.	0.327
37	27 in $4{,}886 \times 10^{7}$ cu. μ, or 48.86 cu. mm.	0.553
17	37 in $5{,}958 \times 10^{7}$ cu. μ, or 59.58 cu. mm.	0.621
27	44 in $6{,}426 \times 10^{7}$ cu. μ, or 64.26 cu. mm.	0.685
12	115 in $5{,}434 \times 10^{7}$ cu. μ, or 54.34 cu. mm.	2.12
11	171 in $7{,}790 \times 10^{7}$ cu. μ, or 77.90 cu. mm.	2.20
13	152 in $6{,}630 \times 10^{7}$ cu. μ, or 66.30 cu. mm.	2.29
9	107 in $4{,}207 \times 10^{7}$ cu. μ, or 42.07 cu. mm.	2.54
28	162 in $6{,}160 \times 10^{7}$ cu. μ, or 61.60 cu. mm.	2.63
6	192 in $5{,}066 \times 10^{7}$ cu. μ, or 50.66 cu. mm.	3.79
4	332 in $6{,}640 \times 10^{7}$ cu. μ, or 66.40 cu. mm.	5.00
Total	1,371 in $68{,}971 \times 10^{7}$ cu. μ, or 689.71 cu. mm.	1.988

* Range, 0.327/cu. mm. to 5.000/cu. mm.; mean, 2.200/cu. mm.; average, 1.988/cu. mm.

Comment.—It is difficult to perceive the significance of these results. An absolutely selective action is apparent in respect to the inferior olivary nuclei, but otherwise the gradient between various regions is slight. The olivary nuclei are adequately and fairly sampled, and their values contrast distinctly with those of other purely nuclear areas such as the dentate, basal cerebral nuclei, and gray matter of the floor of the fourth ventricle. The regions indicated as medullary and pontine reticularis might have shown higher values if more attention had been paid to measuring individual nuclei, but, because the various sections vary in their exact levels, the material is not adequate to permit accumulation of significant data. General inspection of the distribution of lesions in this region makes it doubtful, however, that any other particular nucleus would show such a decided selectivity as the olivary.

Except for the olivary nuclei, the relative intensities in various regions are apparently related principally to the cellularity, lack of myelin, and possibly the vascularity. A preliminary study of the occurrence of the lesions in the various lamina of the cerebral cortex suggested that they might be directly related to the capillary vascularity. The final results do not show direct proportions, although the

general order of the list in Table 7 puts the few areas for which Foley, Kinney, and Alexander (1942) give values for relative capillary vascularity in the human brain into the correct sequence (Table 9).

The curious results in the cerebellum deserve mention. The molecular cortex, which is of very low cellular density and of lower vascularity than the granular cortex, contains a very large number of lesions. The granular layer, on the other hand, is devoid of lesions, with only one or two exceptions in the entire material. This is not due just to the difficulty of distinguishing the cells of the lesions against the background of the cellular cortex, for a special search was made in both thick and thin sections of other patients, as well as those in this group, without yielding any contradiction to this observation. This result alone suggests the importance of some unknown factor other than cellularity or vascularity in the localization of typhus lesions. The large concentration of axons, dendrites, and synapses in the molecularis may be a clue. Typhus is not unique in this preponderance of lesions in the molecular layer, as a similar phenomenon has been observed in Japanese B encephalitis (Haymaker and Sabin, 1947).

TABLE 9.—*Comparison of Intensity of Typhus Lesions with Capillary Vascularity*

Region	Lesions/ Cu. Mm.	Ratio to White Matter	Relative Capillary Densities, μ*
Globus pallidus	5.29	17.7	82
Cerebral cortex	3.38	11.3	51 to 61
Putamen	2.64	8.86	33
Hypothalamus	0.97	3.26	25 to 79

* Values from Foley, I. M.; Kinney, T. D., and Alexander, L.: J. Neuropath. & Exper. Neurol. 1:265, 1942.

Summary.—Quantitative measurements of the intensity of occurrence of the lesions of epidemic typhus in the central nervous system demonstrate only one area that is outstandingly and selectively involved, the inferior olivary nuclei. The other areas may be arranged by the relative intensities of lesions into a series that is divisible into four poorly separated groups. The differences between these groups are apparently directly related to the number of nerve cells and the vascularity and inversely related to the amount of myelin. The high number of lesions in the acellular molecular layer of the cerebellar cortex and the absence of lesions in the cellular granular cortex, however, suggest the operation of other factors, possibly the concentrations of axons, dendrites, and synapses. No difference in the relative distribution of lesions is apparent between lightly as contrasted with heavily involved cases.

COMMENT ON LESIONS OF CENTRAL NERVOUS SYSTEM

Histology.—Detailed studies of the changes in the central nervous system were made many years ago by Benda (1915); Ceelen (1916, 1919); Nicol (1919); Spielmeyer (1919); and Wolbach, Todd, and Palfrey (1922). Little has been added in later years to these accounts and, indeed, we have been unable to find comparable studies in the recent literature. The presence of proliferative nodular lesions associated with blood vessels is regarded as constant and characteristic of epidemic typhus. Other constant lesions which, however, are not regarded as characteristic are perivascular accumulations of macrophages, lymphocytes, and plasma cells and infiltrations of the meninges with similar cells.

The characteristic nodule originally recognized by von Prowazek (1914) and Fraenkel (1914) has been described earlier in this report, as well as by many other authors. Spielmeyer (1919) describes four varieties of the nodule, and this has been confirmed by Wolbach, Todd, and Palfrey (1922). Spielmeyer calls the common or typical nodule the compact lesion. It usually measures 120 to 180 μ in diameter and contains 55 to 75 cells (Allen and Spitz, 1945) which are derived chiefly from vascular endothelium and neuroglia. The role of the neuroglia cell in the formation of the nodules has been emphasized on the basis of examination of human material and experiments in guinea pigs (Wolbach, Todd, and Palfrey, 1922). Allen and Spitz (1945) expressed the belief that the cells were the earliest or small form of oligodendroglial cells, while Spielmeyer (1919) was of the opinion that the ameboid neuroglia played no part. Polymorphonuclear leucocytes, plasma cells, endothelial cells, and rod cells may also enter into the composition of the nodules (Wolbach, Todd, and Palfrey, 1922). According to Allen and Spitz (1945) pleomorphism and karyorrhexis of cells in the nodules are common, and the glial fibers within and adjacent to the nodules are often edematous or show early demyelination. We have been unable to demonstrate demyelination in the Cairo material other than local destruction within the nodules. Spielmeyer called the second variant of the nodule the rosette form. It is small, commonest in the superficial layers of the cerebral and cerebellar cortex, and composed of rod-shaped and sausage-shaped neuroglia cells distributed radially around capillaries. Spielmeyer named the third type of lesion a glial star and described it as consisting of a single or double layer of glial cells surrounding a precapillary and as sometimes resembling a small rosette lesion because of the radial arrangement of the cells. Glial stars are small and commonest in the pons and spinal cord. The fourth variant of the typhus nodule consists of a diffuse increase in neuroglia in areas about 0.1 mm. in diameter either in the molecular layer of the cerebellum or in the superficial layer of the cerebral cortex. This last variant was rare in Spielmeyer's material, and Wolbach, Todd, and Palfrey (1922) were unable to find it in human brains, although they did observe it in the brains of guinea pigs experimentally infected with R. prowazekii. The significance, if indeed there is any at all, of such an arbitrary classification of the variations in appearance of the typhus nodule is not clear.

All pathologists are agreed that capillary and arteriolar damage similar to that found in the skin and other tissues of the body occurs in the nerve tissue of the central nervous system and that vascular lesions can be demonstrated in the nodules provided proper methods such as silver staining and serial sectioning are used. Hemorrhage from injured vessels is usually limited to perivascular spaces, although occasionally it may be extensive enough to destroy adjacent parenchyma. Macrophages containing red blood cells are common in such areas. Schopper (1943) described a rare case in which there was severe meningeal involvement, with thrombosis of the great longitudinal sinus of the meninges and pial veins and massive hemorrhage into the substance of the brain.

In addition to nodular lesions Wolbach, Todd, and Palfrey (1922) have described perivascular accumulations of lymphoid and plasma cells and more unusually macrophages and mast cells which they found most commonly in the basal ganglia, pons, and medulla. These, together with meningitis, they attribute to a general response to infection with typhus rather than to a local response due to localization of rickettsiae, such as is the case with the nodules.

Although not peculiar to typhus, ganglion cell changes have been described by most authors. Wolbach, Todd, and Palfrey (1922) found them in material obtained within two hours of death and therefore thought that they probably occurred during life and were not due to postmortem decomposition. They described neuronophagia in all parts of the cerebrum and cerebellum and chromolysis and axonal reaction in the nuclei in the medulla and midbrain and in the Purkinje cells of the cerebellum. Ganglion cells were observed to have undergone complete destruction in areas involved by proliferative lesions. Satellitosis of ganglion cells also occurred.

Lesions of the type described by Lillie (1941) as microinfarcts in the brains of patients who died from proved Rocky Mountain spotted fever have not previously been observed in epidemic typhus. Golden (1945) saw zones of bland necrosis without cellular reaction in sections from the brain of a Guatemalan who died of epidemic typhus, but it is not certain from the description and illustration that these were the same as Lillie's microinfarcts. Considerable numbers of microinfarcts were present in the Cairo patients, and their similarity with the lesions in Rocky Mountain spotted fever seems established. However, the nature of these so-called microinfarcts is not clear. It has not been proved that they are true infarcts, and a relation to affected arterioles could not be demonstrated in the Cairo patients. The round bodies which appear in the lesions are probably axons, as they take up silver faintly and do not stain in the manner of myelin. Detailed histochemical studies of the general and specific reactions of these lesions have not yet been made, and so final decision as to their nature and significance must be postponed.

Specificity of Nodules.—Typical nodules in the brain and spinal cord are usually regarded as being highly specific of the typhus group of rickettsial infections—epidemic typhus, murine typhus, Rock Mountain spotted fever, scrub typhus. While this is true in most cases, nevertheless, similar nodular lesions may occur in such widely different diseases as Western equine encephalitis, St. Louis type of encephalitis, epidemic encephalitis of Japan (Wolbach, 1948), Chagas' disease, malaria, toxoplasmosis (Allen and Spitz, 1945), and typhoid fever (Wolbach, Todd and Palfrey, 1922).

The morphologic demonstration of rickettsiae in the endothelial cells of blood vessels in Giemsa-stained paraffin sections of the brain was accomplished in both human and guinea pig tissues by Wolbach, Todd and Palfrey (1922). They also saw them in neuroglia cells, as well as between the cells in guinea pigs, observations which were difficult to make in human material because of the presence of fine granules in the neuroglia cells.

Topography of Lesions.—The only other study that gives detailed information about the distribution of lesions in the central nervous system is that of Wolbach, Todd, and Palfrey (1922). They discovered lesions in every one of 37 brains examined, whereas in our material, which consisted of 29 brains, lesions were found in 26. The distribution of lesions is essentially the same in the two series, although minor differences are apparent when their findings are compared with ours (Table 7). According to Wolbach, Todd, and Palfrey (1922), both proliferative lesions and perivascular infiltrations are commonest in the medulla and especially in the olivary nuclei. This is true in the Cairo cases and had been observed long ago by both Ceelen (1916) and Nicol (1919). Wolbach, Todd, and Palfrey (1922) found also that the pons, especially the gray matter (nuclei pontis), the midbrain, and

basal ganglia showed numerous nodular lesions and perivascular infiltrations. Next in order of frequency of proliferative nodular lesions were the central gray matter of the fourth ventricle, just below the ependyma; the gray matter of the cerebral cortex in which the middle group of ganglion cells in layers II to V were most markedly affected; Ammon's horn, and the cerebellum, in which the order of involvement was the molecular layer and dentate nucleus, Purkinje cell layer, granular layer, and white matter. Of the cortical areas the parietal lobe contained the most nodules and the occipital lobe the fewest. Although the choroid plexus was examined in every instance, no lesions were found, which is in contrast to the findings of Ceelen (1916) and ourselves. The chief difference between the two series is that Wolbach, Todd, and Palfrey always found numerous lesions at the decussation of the pyramidal tracts, whereas only a few were discovered in the Cairo patients. In the two series, white matter and the internal capsule were rarely attacked. Focal infiltrations and perivascular collections of mononuclear cells in the piarachnoid were practically constant in the Warsaw patients, being most marked over the cerebellum, pons, and medulla, and reaching a maximum at two weeks. Nodules were rarely encountered. A similar type of meningitis was also common in the Cairo patients. Allen and Spitz (1945) reported that cortical white matter was usually spared and that the following areas were involved in from 79 to 87% of their cases: gray cortex, pons, medulla, basal ganglia, cerebellum, and spinal cord. They also observed mononuclear cell infiltration of the meninges in 73%.

Clinical Pathological Correlation.—The only report known to us that correlates clinical and anatomical findings in the central nervous system is that of Wolbach, Todd, and Palfrey (1922). They found that the severity of the skin rash during life corresponded fairly well with the degree of cerebral involvement found at postmortem examination. Ceelen (1916) also described this phenomenon. There was also a correlation between the severity of the mental and motor disturbances during life and the distribution of cerebral lesions after death. Motor disturbances, such as twitching, trismus, and cerea flexibilitas, were associated with extensive involvement of the cerebral cortex. However, marked mental symptoms were accompanied more with marked general involvement of the brain than with localization in any particular areas. They noted that marked cardiac disturbance was definitely associated with extensive lesions in the medulla, which they thought was partly due to the effects of capillary hemorrhages in this region. They also suggested that similar perivascular hemorrhages might be the cause of respiratory symptoms. However, in a later paper Wolbach (1948) wrote that "in spite of the fact that the medulla is a region of heavy distribution of 'nodular' lesions, no evidence of an effect upon the 'vital centers' has been proved."

Histogenesis.—The most complete account of the histogenesis of the typhus nodule in the brain is that of Wolbach, Todd, and Palfrey (1922), who studied the development of the lesions in experimentally infected guinea pigs from the 1st to the 24th day and in human brains before the 10th day of the disease. They found that the earliest recognizable lesion, which was present in guinea pig brains on the first or second day of fever, occurred in the capillaries and precapillaries and consisted of swelling of the lining endothelial cells. Various degenerative changes were present in both cytoplasm and nuclei, and the swelling was sometimes so great as to cause occlusion of the lumen of the affected vessel. Small platelet and fibrin thrombi

formed, and small perivascular hemorrhages were constant about vessels of precapillary size. The next stage consisted of the appearance around injured vessels of mononuclear cells of two types, perivascular neuroglia cells and macrophages. The latter appeared to come from the endothelium. The reaction of the neuroglia cells occurred simultaneously with that of the endothelium, and together they formed the nodules. Acquisition of new cells accounted for the increase in size of the nodules. Polymorphonuclear leucocytes were invariably present in small numbers in the early stages, and sometimes, especially if there was necrosis of brain tissue, they predominated. Lymphoid and plasma cells were usually absent. In adjacent brain tissue, radially arranged rod cells (elongated neuroglia cells with granular cytoplasm) surrounded the nodules. Satellitosis and neuronophagia of ganglion cells were also observed.

The various stages, from the earliest lesions to full blown ones, were usually to be found in the same brains and were said to persist for as long as the eighth week (Nicol, 1919). Healing usually occurred by degeneration and migration of the cells composing the nodules, which were then sometimes replaced by small amounts of fibrillary neuroglia. Scars have been found in the brain three to four months after the onset of typhus (Nicol, 1919). Experiments performed by Wolbach, Todd, and Palfrey (1922) indicated that rickettsiae might survive in the brains of guinea pigs after the temperature became normal. Early lesions were found in apparently recovered animals.

PERIPHERAL NERVES AND GANGLIA

WILLIAM B. WARTMAN, M.D.

RÉSUMÉ

SUFFICIENT material was not available in the Cairo cases for adequate study of possible changes in peripheral nerves and ganglia. The clinical occurrence of neuritis with varying degrees of muscle wasting, paralysis, sensory disturbances and eventual recovery has been described in six German soldiers by Grubmüller (1943). Involvement of the following combinations of nerves was detected: the two ulnars; left ulnar, left upper brachial plexus, and right ulnar; the two upper brachial plexuses; left median. D'Ignazio, Lombardi, and d'Archangelo (1940) reported polyneuritis developing 4 to 30 days after defervescence in European patients who were stricken while in Abyssinia. The neuritis persisted for about one month, and only one patient achieved a complete cure. Sangiovanni (1940) commonly found clinical evidences of neuritis of the peroneal, ulnar, and auditory nerves and thought that it might be of toxic origin and possibly due to drugs. Herzog (1936) made an extensive study, using many histological techniques, of the alterations which occur in the vagus nerve and the sympathetic ganglia and has written a well-documented and illustrated report of his results. The material was obtained from 80 fatal cases in an epidemic of typhus in Chile during 1932-1935. The vagus nerve and ganglion nodosum and the superior cervical sympathetic ganglion were examined in all instances and the stellate ganglion in a single instance. In a few instances specimens were obtained bilaterally. Characteristic vascular lesions including thrombi and nodular accumulations of cells were found in 87% of sympathetic ganglia and in 84% of vagus nerves, and there was no difference between the right and left sides of the body. Venous hyperemia, marked dilatation of the sinuses of Ranvier, and

small scattered hemorrhages were constant features. About 10% of patients showed diffuse ganglionitis instead of the nodular variety, and this was especially apparent in the ganglion nodosum. Degenerative changes proportional to the extent and severity of inflammation were seen in the ganglia but not in nerve fibers. In one instance rickettsiae were said to have been discovered in capillary endothelium in the superior cervical ganglion. Most of the specimens were from patients who died at the end of the first or second week of their illness, although five of them died at the end of the fifth week. Pathological changes were most marked during the first week, and healing was observed as early as the third week.

Falin (1945) has given an account of the changes undergone by the terminal portions and endings of the peripheral nerves in the skin and epiglottis. These structures are markedly but irregularly affected in epidemic typhus. Nerve fibers may show degenerative changes, as may the Meissner's and genital corpuscles of the skin and the end-bulbs of Krause in the glans penis. Usually the normal structure is retained, although globular thickening (Kugelphänomen) of the ends of terminal filaments may occur. Falin was of the opinion that most of the nerves involved belonged to the vegetative or sympathetic nervous systems.

Wolbach, Todd, and Palfrey (1922) examined the Gasserian ganglion from 26 patients and discovered lesions due to epidemic typhus in 15. These lesions included characteristic capillary lesions; proliferative lesions in nerve trunks, which were indistinguishable from the "compact" type of lesion in the brain; perivascular accumulations of mononuclear cells, and degenerative changes in ganglion cells attended by swelling and proliferation of the capsular cells. Complete disappearance of ganglion cells leaving a residuum of Nissl granule dust in spaces nearly filled by proliferated capsular cells was frequent in ganglia with extensive lesions. They noted that this later change was not unlike that of rabies, although less striking. A diffuse infiltration by lymphocytes, plasma cells, and macrophages of considerable areas of the ganglion was common, and such areas usually included one or several degenerated ganglion cells with capsular proliferation. Perivascular accumulations of similar cells were discovered in the nerve trunks and the arachnoid investments of the ganglion.

ORGANS OF SPECIAL SENSE

WILLIAM B. WARTMAN, M.D.

RÉSUMÉ

THE ORGANS of special sense were not available for pathological examination in the Cairo cases. The literature contains the following information.

EYES

Coincidental with the appearance of the rash, the conjunctival blood vessels are said to become dilated and congested, and petechiae appear. These signs tend to increase with the progress of the eruptive stage until the beginning of defervescence (Wolbach, Todd, and Palfrey, 1922; Yeomans, 1948; Ling, 1929; Guerra, 1940). Photophobia is frequently present during the critical period of the disease (Yeomans, 1948). In severe and usually fatal cases the corneal epithelium is said to swell and the surface layers to be shed. This usually takes place at the periphery but may extend and involve as much as one-fourth of the cornea (Guerra, 1940). About the

sixth day of the disease Guerra (1940) observed inflammation and edema of the optic nerve which increased for a period of about one week and usually subsided after the crisis, although occasionally persisting for as long as a week after the fever. Rarely, small white spots appeared on the retina, as in albuminuric retinitis. According to Yaluff and Verdaguer (1942) minute white spots on the retinal vessels are present in 80% of cases of epidemic typhus and are diagnostic. They describe them as being refractile, with well-defined edges, and sometimes surrounded by a red zone. They occur either on vessels or at some distance away and may be scattered over a wide area, especially at the periphery where they are likely to appear first. They are located in the retina or deep in the choroid. They are said to appear at the end of the febrile period or sometimes earlier, to last for 8 to 20 days, and not to produce symptoms.

According to Avtsyn (1943), the conjunctival petechiae constitute a conjunctival exanthema. They were present in 94% of patients who died from typhus in Moscow, being absent only when death occurred late or was due to a complication such as pneumonia. However, they were present in only 27% of living patients. Microscopic examination showed that they resulted from specific vascular lesions of capillaries and arterioles with thrombosis.

EARS

Roaring in the ears, followed by deafness and tinnitus, is frequently an early symptom (Yeomans, 1948) and may persist with gradual improvement into convalescence without clinical evidence of middle ear disease (Wolbach, Todd, and Palfrey, 1922). Otitis media due probably to secondary bacterial infection is also seen (Wolbach, Todd, and Palfrey, 1922). In recent epidemics the incidence of ear symptoms has been as high as 80%, usually becoming evident on the second or third day. About 15% of patients of one series had suppurative middle ear disease coming on about the third week, and in some of the patients mastoiditis developed. The prevailing organisms were hemolytic streptococci and staphylococci (Seiferth, 1944). Another author observed changes in the tympanic membrane in 22 of 50 patients (Sangiovanni, 1940). It was opaque in 17 and inflamed in 7 patients. One patient was operated on for mastoiditis. Clinical evidence of auditory neuritis was also observed in these patients.

MISCELLANEOUS PATHOLOGICAL FINDINGS

IN THE Cairo patients the serous membranes were relatively dry and sticky in dehydrated patients but normal in others. Slight accumulations of clear straw-colored fluid were noted in the pleural and pericardial cavities. Wolbach, Todd, and Palfrey (1922) noted similar changes and, in addition, dusky injection of tendon sheaths in the ankles. Some patients are said to have a greasy peritoneum (Aschoff, 1915). According to Wolbach (1948), sterile or infective proliferative pericarditis and pleuritis do not occur in epidemic typhus.

GENERAL COMMENT

WILLIAM B. WARTMAN, M.D.

ALTHOUGH many of the interesting and controversial features of the disease have been discussed in other sections of this paper, there are some general

features of the pathological physiology and anatomy of epidemic typhus that deserve special comment, if only to indicate the need for further study.

SPECIFICITY OF LESIONS

Everyone seems agreed that no single histological lesion is pathognomonic of epidemic typhus. The combination of vasculitis, hyaline thrombi, nodular and diffuse accumulations of mononuclear cells, myocarditis, myositis, and nodules in the central nervous system is probably diagnostic of rickettsial infection. However, it must not be forgotten that occasionally viral, bacterial, or parasitic infections may produce similar lesions. The finding of rickettsiae in vascular endothelium is strong evidence in favor of typhus, but the difficulty of identification of micro-organisms in human tissues is great. Actual isolation of the rickettsiae is needed for positive diagnosis, but in practice it is usually sufficient to evaluate the pathological findings in the light of the clinical, laboratory, and epidemiological features of the disease.

It is even more difficult and often impossible to distinguish epidemic typhus from Rocky Mountain spotted fever and scrub typhus on the basis of an objective study of the lesions, due, no doubt, to the many features this disease group has in common. The comparative pathology of the three groups of diseases has been studied by Allen and Spitz (1945) and Wolbach (1948, 1950). A summary of the differential features described by them follows.

Vascular lesions are least severe in scrub typhus and severest in Rocky Mountain spotted fever, in which the media may be directly injured in contrast to both epidemic and scrub typhus, in which it is almost always spared. This involvement of the media leads to necrosis of arteries and veins in the skin and subcutaneous tissues and accounts for the predominance of necrotic lesions in Rocky Mountain spotted fever and their occurrence independently of pressure stasis. Characteristically they are found in scrotum, prepuce, fingers, toes, and ear lobes. In epidemic typhus necrosis of skin and subcutaneous tissues occurs only in regions subjected to pressure. Petechiae, which are related to the degree of capillary injury, are most abundant in Rocky Mountain spotted fever, abundant in epidemic typhus, and rare in scrub typhus. The skin eruption is centrifugal in epidemic typhus and scrub typhus but centripetal in Rocky Mountain spotted fever. There is a correlation between the severity of vascular lesions and the onset of the rash, which appears at about the 3rd day in Rocky Mountain spotted fever, from the 3rd to the 7th day in epidemic typhus, and not until the 7th to the 14th day in scrub typhus. An eschar is present at the site of primary inoculation in most cases of scrub typhus, fièvre boutonneuse, and South African tick-bite fever. In the central nervous system, nodules are constant in epidemic and scrub typhus, with the earliest lesion usually about capillaries, whereas in Rocky Mountain spotted fever it may be about larger vessels and cause small infarcts, which have not been observed in scrub typhus. Meningeal and perivascular infiltration is most constant and greatest in scrub typhus. The descending order of the degree of involvement of the heart is scrub typhus, epidemic typhus, and Rocky Mountain spotted fever. Diffuse infiltrations of mononuclear cells are most common in scrub typhus, which is of some importance in the lungs where such cellular accumulations both inside and outside capillaries may compress and block alveolar capillaries. This is marked in scrub typhus but is negligible in epidemic typhus. Interstitial pneumonia probably due to direct action of the rickettsiae occurs in scrub typhus, to a less degree in Rocky Mountain spotted fever and epidemic

typhus. The lymph nodes in a fair percentage of cases of scrub typhus but in neither of the other diseases show small foci of necrosis. Swelling, proliferation, and serous exudation of the mesothelium of the pericardium and pleura unassociated with bacterial infection occur in scrub typhus.

Q fever, rickettsialpox, and other human rickettsial diseases differ so much from epidemic typhus both clinically and pathologically that they are usually easily distinguished.

CIRCULATORY FAILURE

Circulatory failure may occur during the acute stages of epidemic typhus and is often indicative of impending death. The extremities become cold and cyanotic; the arterial blood pressure drops to low levels, and the arterial pulse becomes feeble and rapid (Yeomans, 1948; Wolbach, Todd, and Palfrey, 1922; Wolbach, 1948, 1950). Shock, hypochloremia, azotemia, lowered plasma volume, and increase in acid anions have been reported (Tierney and Yeomans, 1946). This type of failure has been called "peripheral vascular failure" (Yeomans, 1948), and it is interesting to note that it has been described more frequently and has been given more attention in recent epidemics (Yeomans, 1948; Woodward and Bland, 1944; Aschenbrenner, 1943; Siedek, Kasperczik, and Fanta, 1943; Robbers, 1943; Laurentius, 1943; Randerath, 1943; Schopper, 1943; Sturm, 1942) than in previous epidemics (Wolbach, Todd, and Palfrey, 1922). Congestive heart failure is very rare either in the acute stages of the disease or during convalescence, except in moribund patients, and is not relieved by digitalis (Yeomans, 1948; Wolbach, Todd, and Palfrey, 1922). The electrocardiogram frequently shows low voltage, inversion of T waves, depression of ST segments, and an increase in PR interval, but these changes are not specific, as they are known to occur in other acute infections. They rarely persist longer than four weeks (Yeomans, 1948). Auricular fibrillation may also occur (Wolbach, Todd, and Palfrey, 1922).

The explanation of this peripheral vascular failure is unknown. It has been attributed to extensive involvement of capillaries of the skin and other organs (Wolbach, 1948; Randerath, 1943), to injury of the vasomotor centers in the brain (Laurentius, 1943; Randerath, 1943; Sturm, 1942), to injury of the adrenal cortex with resulting adrenal insufficiency (Golden, 1945; Allen and Spitz, 1945), to involvement of the lungs and kidneys (Allen and Spitz, 1945), and to direct myocardial injury (Schopper, 1943; Allen and Spitz, 1945). Most recent students of epidemic typhus have favored the first 2 explanations or a combination of them and have minimized the importance of the myocarditis, chiefly because it is sometimes difficult to prove a good correlation between the clinical signs of circulatory failure and the pathological findings in the myocardium and because of the lack of convincing histological evidence of degenerative changes in the myocardial fibers. However, in view of the constant and often severe myocarditis in the fatal Cairo cases, we do not regard this factor so lightly.

RENAL FUNCTION

Clinical manifestations of altered renal function include the following: albuminuria, which is of varying degree and almost constant during the febrile period (Wolbach, Todd, and Palfrey, 1922; Yeomans, 1948, and others); large numbers of granular casts in patients with nitrogen retention (Yeomans, 1948); red blood

cells in the urinary sediment but no gross hematuria (Yeomans, 1948); azotemia with elevated concentrations of blood nonprotein nitrogen, urea nitrogen, and creatinine (Yeomans, Snyder, Murray, Ecke, and Zarafonetis, 1945; Murchison, 1862; Wagner, 1920; Walther, 1942; Sturm, 1942; Wetzel, 1940; Aschenbrenner, 1944; Woodward and Bland, 1944); oliguria (Yeomans, 1948; denied by Wolbach, Todd, and Palfrey, 1922); hypochloremia (Tierney and Yeomans, 1946; Julliard and Henaff, 1939; denied by Sturm, 1942; Aschenbrenner, 1944); increase in acid anions of the blood (Tierney and Yeomans, 1946); depression of the albumin fraction and elevation of the globulin fraction in the presence of normal total serum proteins (Tierney and Yeomans, 1946), and a rise in serum bicarbonate (Woodward and Bland 1944; denied by Tierney and Yeomans, 1946). A clinical hepatorenal syndrome with nitrogen retention and jaundice has been described (Koranyi and Varga, 1943).

Azotemia in epidemic typhus has been extensively studied by Yeomans, Snyder, Murray, Ecke, and Zarafonetis (1945), and, since their conclusions are adequately supported by careful clinical observations and well-controlled laboratory data, we will review them briefly. They discovered azotemia in about 52% of patients studied by the United States of America Typhus Commission in Cairo during 1943 and 1944. The correlation between clinical severity of the disease and azotemia was striking, and every patient whose blood was examined after death had an elevated nonprotein nitrogen regardless of age or other demonstrable factors. Although the appearance of azotemia was of serious prognostic significance, patients who survived showed no evidence of renal impairment at the end of several months. These authors describe three types of renal failure. The first and commonest is probably caused by a combination of excessive destruction of body protein and high fever with dehydration, producing a low output of highly concentrated urine. The azotemia is never very high, and restoration of adequate hydration frequently results in its prompt disappearance. The second type of renal failure is characterized by the development of azotemia (nonprotein nitrogen in excess of 150 mg. per 100 cc. of blood) without accompanying dehydration, hemoconcentration, fall in blood pressure, or decrease in urine volume. It usually appears between the 8th and 11th days, and the patients have a stormy course, with intractable hiccough, incontinence or retention of urine and feces, and profound stupor. Death often ensues, preceded by high fever, ever increasing nitrogen retention, and oliguria. The third type occurs in severe cases and is associated with a rapid fall in blood pressure, often within a few hours, and evidence of renal insufficiency. When the systolic blood pressure falls below 70 mm. Hg, peripheral vascular collapse may ensue. These authors conclude on the basis of their own studies that the development of nitrogen retention in epidemic typhus is most likely due to extrarenal factors, such as greatly increased protein catabolism, dehydration, and reduction of arterial blood pressure, rather than to primary renal damage brought about by direct action of the rickettsiae.

The questionable existence of a true diffuse glomerulonephritis attributable to the direct or indirect action of rickettsiae is discussed in the section on the urinary tract. Some authors have found it in a high percentage of cases (Dawydowskie, 1924; Herzog, 1935; Caffarena, 1937; Wetzel, 1942; Schopper, 1943; Randerath, 1943; Allen and Spitz, 1945) and others rarely (Ceelen, 1919; Wolbach, Todd, and Palfrey, 1922; Wagner, 1920; Munk, 1925; Aschenbrenner, 1944; Julliard

and Henaff, 1939; Report of National Research Council, 1953). The cases studied by Allen and Spitz are included in this report, and we have been unable on reexamination of the microscopic slides to confirm their finding of a high incidence of the intracapillary form of acute diffuse glomerulonephritis; we are of the opinion that acute diffuse glomerulonephritis has not been convincingly demonstrated to occur in many cases of epidemic typhus.

On the basis of autopsy studies Golden (1945) has suggested that a lower nephron nephrosis may occur in some patients. This view needs confirmation, since published clinical studies and other pathological reports, including our own, have not shown such a lesion.

HYPERSENSITIVITY

Because of the character of certain of the tissue reactions in the typhus group of diseases, such as fibrinoid degeneration of collagen, necrosis of lymph nodes and spleen, the predominance of basophilic macrophages and plasma cells, and the so-called acute diffuse glomerulonephritis, Allen and Spitz (1945) have suggested that rickettsiae may have hyperergic effects. However, such findings are not pathognomonic of hypersensitivity and may occur either singly or in combination in many diseases in which hypersensitivity is not known to be active. Fibrinoid degeneration of collagen, in our experience, is not common in epidemic typhus, and necrosis of lymph nodes and spleen is absent (Wolbach, 1948). The occurrence of true acute glomerulonephritis seems questionable, as indicated in the discussion of the kidney lesions. Other authors have also expressed the opinion that hypersensitivity may play a part in epidemic typhus, but in no instance has objective proof been brought forward to support this belief. Abrikosov (1941) suggested that the changes in the arteriocapillary system might be caused by penetration of the endothelium by rickettsiae and subsequent allergic sensitization. Bucco (1951) attributed the meningitis and choroiditis to allergic shock resulting from the interaction of antigens and antibodies. Walther (1942) observed patients in whom slight fever accompanied by urticaria recurred during convalescence and attributed this syndrome to an allergic state but without giving the evidence for this opinion. He also believed that agglutinins and other protective substances in typhus were greatly increased after the disease had subsided.

PATHOGENESIS

The louse transmission of epidemic typhus was proved by Nicolle, Comte, and Conseil (1909), and Ricketts and Wilder (1910) first observed the micro-organisms in the blood of patients and in the intestinal contents of infected lice. A decade later Wolbach, Todd, and Palfrey (1922) proved "that the virus of typhus and Rickettsia prowazekii were inseparable and therefore the same." As a result of the labors of many scientists, it is now known that lice (Pediculus humanus corporis) become infected by feeding on humans suffering from the disease. The rickettsiae invade the lining of the gut of the lice and there multiply, eventually causing the cells to rupture and release the rickettsiae into the intestinal contents. They are then passed in the feces, which remain infective for long periods of time, and it is thought that infection in the human results from scratching the feces into the skin rather than directly from the bite of the lice. Contamination of mucous membranes and inhalation of infective material suspended in the air may also provide portals of entry for rickettsiae (Kohls, 1948).

It seems likely that the rickettsiae are introduced or pass directly into the blood stream, since neither a primary lesion, lymphangitis, nor lymphadenitis are features of epidemic typhus. Experimental investigation of this point should be possible and is desirable. From experiments in guinea pigs and at biopsy on examination of specimens obtained from humans in all stages of the disease (Wolbach, Todd, and Palfrey, 1922), it has been established that after entry into the body the rickettsiae localize and multiply in the intimal endothelium of capillaries and then spread centripetally to larger blood vessels. The localization in capillaries explains the widespread distribution of the lesions. The demonstration of micro-organisms in the endothelial cells of blood vessels and the failure to find them elsewhere are highly suggestive that both the lesions and their site are determined by the presence of the rickettsiae.

Lesions are produced in and immediately adjacent to blood vessels and are responsible for the characteristic pathological findings and clinical manifestations. Because of the presence of the causative agent within vascular endothelium, proliferation of these cells precedes exudative responses or necrosis (Wolbach, 1950). From studies of specimens of the skin of humans removed for biopsy and examination of the skin and brain of experimentally infected guinea pigs (Wolbach, Todd, and Palfrey, 1922) and the tissues of white mice (Avtsyn, 1944; Siegert, 1950) and rats (Siegert, 1950), it is known that swelling and proliferation of vascular endothelium are the earliest recognizable changes and are present in the skin on the first day of the eruption or perhaps earlier. Vasculitis and thrombosis, which with the endothelial proliferation are responsible for many of the clinical features of typhus, are present by the fifth day of the eruption. Propagation of thrombi, as well as spread of the vascular lesions, is centripetal. Rickettsiae have been shown to enter into and multiply in the large mononuclear cells that accumulate about blood vessels (Wolbach, 1950). The exact nature of these mononuclear cells is in dispute, some believing that they are of mesenchymal origin (Wolbach, 1948, 1950) and others that they are derived from lymphoblasts (Allen and Spitz, 1945). All are agreed that they occur in both nodular and diffuse accumulations.

Detailed information about the development of the lesions is available only for the skin of humans and guinea pigs (Wolbach, Todd, and Palfrey, 1922); the central nervous system of guinea pigs, white mice, and rats (Wolbach, Todd, and Palfrey, 1922; Siegert, 1948), and the lungs of white mice (Avtsyn, 1944). The origin and development of nodules in the central nervous system are the same as in the skin. The initial reaction is proliferative and involves mononuclear cells which are probably of mesenchymal origin. Neuroglial cells of various types soon appear, and finally neuroglial scars may be produced. Direct involvement of ganglion cells in the cerebral and cerebellar cortices and in many nuclei occurs and may be the cause of some of the nervous manifestations. Despite the fact that nodular lesions are common in the medulla, there is no proof of an effect on "vital centers" (Wolbach, 1948).

Correlation of lesions and clinical manifestations seems best in the skin, where the presence and time of onset of the rash, petechiae, and necrosis are dependent on the severity and distribution of vascular lesions. A good correlation can also often be shown in the central nervous system, but in other organs, notably the heart, kidneys, liver, and adrenal gland, it is more difficult to demonstrate. It seems likely,

although by no means proved, that many of the clinical manifestations are due to widespread involvement of capillary endothelium rather than to local involvement of single organs.

Information concerning persistence and healing of lesions is meager. According to Wolbach (1948), they may persist in the brain of the guinea pig and even increase in size after fever has subsided. In human brains they have been found eight weeks after recovery, the final stage being a minute neuroglial scar (Wolbach, 1948). Abrikosov (1941) reported that during convalescence the proliferative angiitis and the nodules in the brain and other organs disappeared completely by a process of degeneration and absorption of the proliferated cells and that afterwards the integrity of the arterial wall was restored. Randerath (1943) found that the exanthema could be detected microscopically as long as 23 to 37 days but that the nodules had disappeared by the 31st day.

Experiments in guinea pigs indicate that the incubation period of the uncontaminated virus is seven days (Nicolle, Conseil, and Conor, 1912; Anderson, 1914; Wolbach, Todd, and Palfrey, 1922), and clinical experience indicates that the same is probably true for man (Mathew, 1936).

Secondary bacterial infections are so common in epidemic typhus that they may be considered a regular part of the disease. Although the infective agents are usually said to be hemolytic streptococci, staphylococci, or pneumococci, we have been unable to find a report of a satisfactory study of the etiology of these infections. All three micro-organisms, or others, could account for the development of pneumonia, pleurisy, empyema, lung and brain abscesses, meningitis, pericarditis, parotitis, and sialadenitis. Erysipelas, phlegmons, and multiple abscesses of the skin are probably the result of streptococcal and staphylococcal infection, as are otitis media and mastoiditis. It is also possible that the acute diffuse glomerulonephritis reported in some epidemics may be the result of streptococcal infection. This possibility has been largely overlooked in the literature. Laryngeal and tracheal diphtheria was common among patients observed by German physicians in World War II. One of the Cairo patients showed an associated fungus infection of unidentified type in the lungs. Although many of the Cairo patients had schistosomiasis, it seems likely that infection occurred before the onset of typhus and was not dependent upon it.

From this discussion of the pathogenesis of epidemic typhus, it is apparent that our information is by no means complete and that experimental studies of the sort which Madsen (1937) and Fenner (1948) have made of the acute infectious diseases and of the acute exanthemas are needed in order to confirm the available evidence, which is practically all circumstantial, and to supply new information about the ways in which the infectious agent of epidemic typhus spreads in the body and produces lesions.

SUMMARY

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THIS PAPER describes the lesions encountered in an epidemic of typhus which occurred in Cairo during 1943-1945. The findings have been compared to those observed by Wolbach, Todd, and Palfrey in the Warsaw epidemic of 1918 and by others since that time in order to bring together in one report all the known facts about the pathology of epidemic typhus.

The patients studied in the Cairo epidemic were Egyptians between the ages of 10 and 70 years. Some of them were undernourished, but there was no clinical or pathological evidence of avitaminosis, and some had clinically inactive schistosomiasis. The patients were admitted to the hospital during the first 2 weeks of their disease, and the clinical diagnosis of louse-borne typhus was confirmed in many cases by the Weil-Felix and complement fixation tests (Tierney and Yeomans, 1946). In some instances rickettsiae were recovered from blood or from normal lice fed on the patients, and each strain isolated showed the characteristics of louse-borne typhus (Yeomans, Snyder, Murray, Ecke, and Zarafonetis, 1945). Two patients were given paraaminobenzoic acid, without clinical or pathological effects. The other patients received no specific antityphus treatment, and none had been vaccinated against typhus. In 14 patients special efforts were made to reduce secondary bacterial infection by using sulfonamides and penicillin when necessary. This paper and the reports of the United States of America Typhus Commission on the clinical and laboratory features constitute one of the few comprehensive accounts of an epidemic of typhus in Egypt or, indeed, in any tropical country (Kamal and Messih, 1943). It seems likely, in view of the discovery of antibiotics that may be effective in the treatment of the disease and of the development of satisfactory vaccines, that there may never again be a similar opportunity to study an epidemic of typhus which has not been significantly modified either by treatment or by complicating infection.

The lesions discovered in the Cairo patients were essentially the same as those described in other epidemics in different parts of the world and in experimental animals. The wide dissemination of vascular and other lesions in the skeletal muscles, which was well illustrated by the frequent involvement of the muscles of the tongue, was more apparent in Cairo cases than in others, probably because abundant material was available for microscopic examination. Evidence was obtained by the demonstration of rickettsia-like bodies in sections of the lungs, which suggests, but does not prove, that a true rickettsial pneumonia may exist. Interstitial orchitis and prostatitis of a type not ordinarily seen in other infectious diseases was also observed.

New information has been obtained concerning the topography of lesions in the central nervous system and the effect of the duration of the illness on the intensity of the reaction. So-called microinfarcts have been demonstrated for the first time in the brains of patients who died from epidemic typhus.

Glomerulonephritis did not occur in the Cairo patients, and a review of published articles has led to the conclusion that its occurrence has not been proved. The bulk of the evidence supports the idea that renal failure in epidemic typhus is probably due to extrarenal factors, such as increased protein catabolism, dehydration, and reduction of arterial blood pressure, rather than to primary renal damage brought about by direct action of the rickettsiae. We have not been able to convince ourselves that lower nephron nephrosis occurs.

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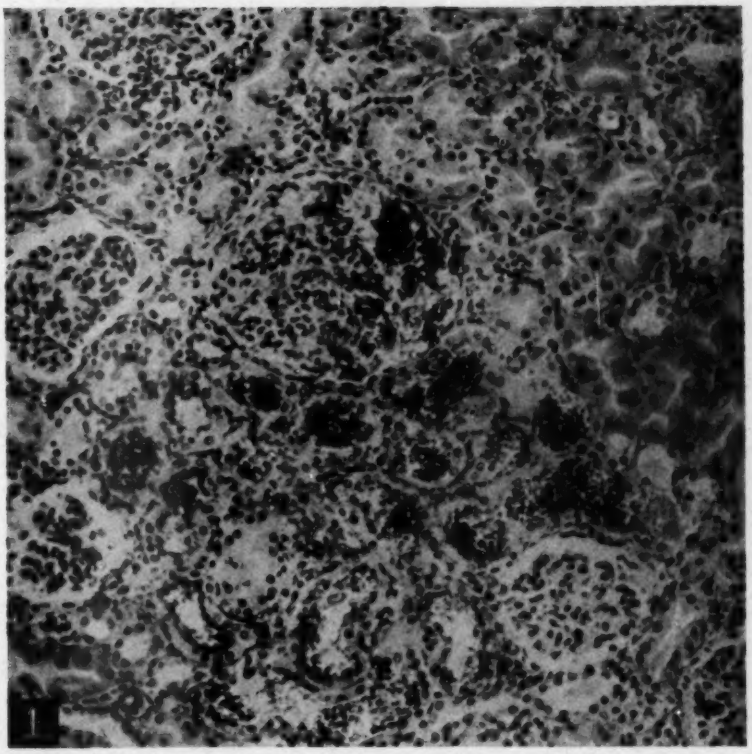

Fig. 1 (Case 16).—Hemorrhage in the subcapsular space of a renal glomerulus, with extension into proximal tubules. No morphologic lesion of tuft; × 142.

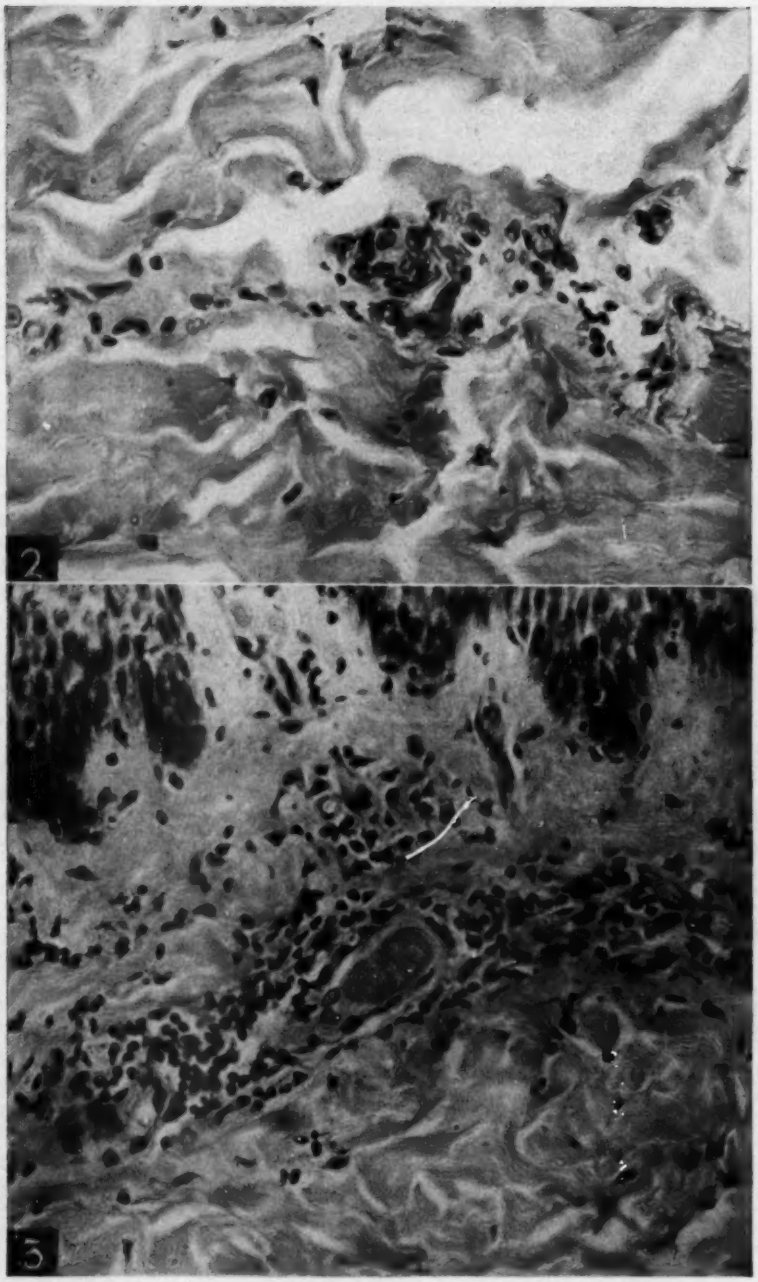

Fig. 2 (Case 21).—Capillary in dermis showing swelling and proliferation of endothelium to point of occlusion. Slight perivascular accumulation of mononuclear cells; × 430.

Fig. 3 (Case 8).—Finely granular capillary thrombus, with pericapillary collection of mononuclear cells in dermis; × 312.

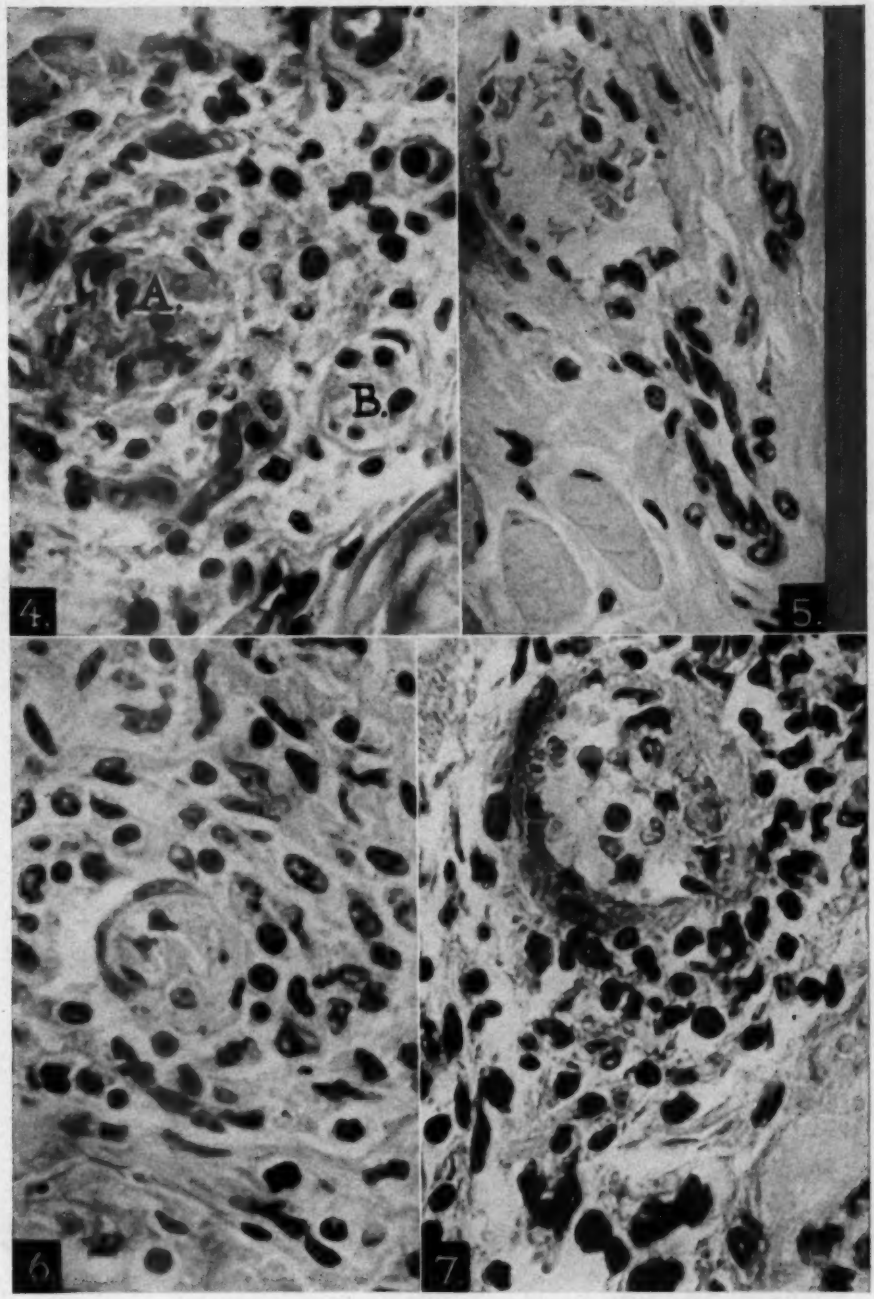

Fig. 4 (Case 33).—Necrosis and thrombosis of dermal capillary (*A*), with many perivascular mononuclear cells of different types. A small nerve (*B*) is included; × 730.

Fig. 5 (Case 40).—Swelling and proliferation of endothelium of capillary in tongue; × 730.

Fig. 6 (Case 40).—Occlusion of capillary in tongue by swelling and necrosis of endothelium. Pronounced exudation of mononuclear cells in the surrounding tissues; × 875.

Fig. 7 (Case 5).—Myocardium. Focal swelling and necrosis of capillary endothelium and perivascular infiltration of mononuclear cells with hyperchromatic nuclei; × 875.

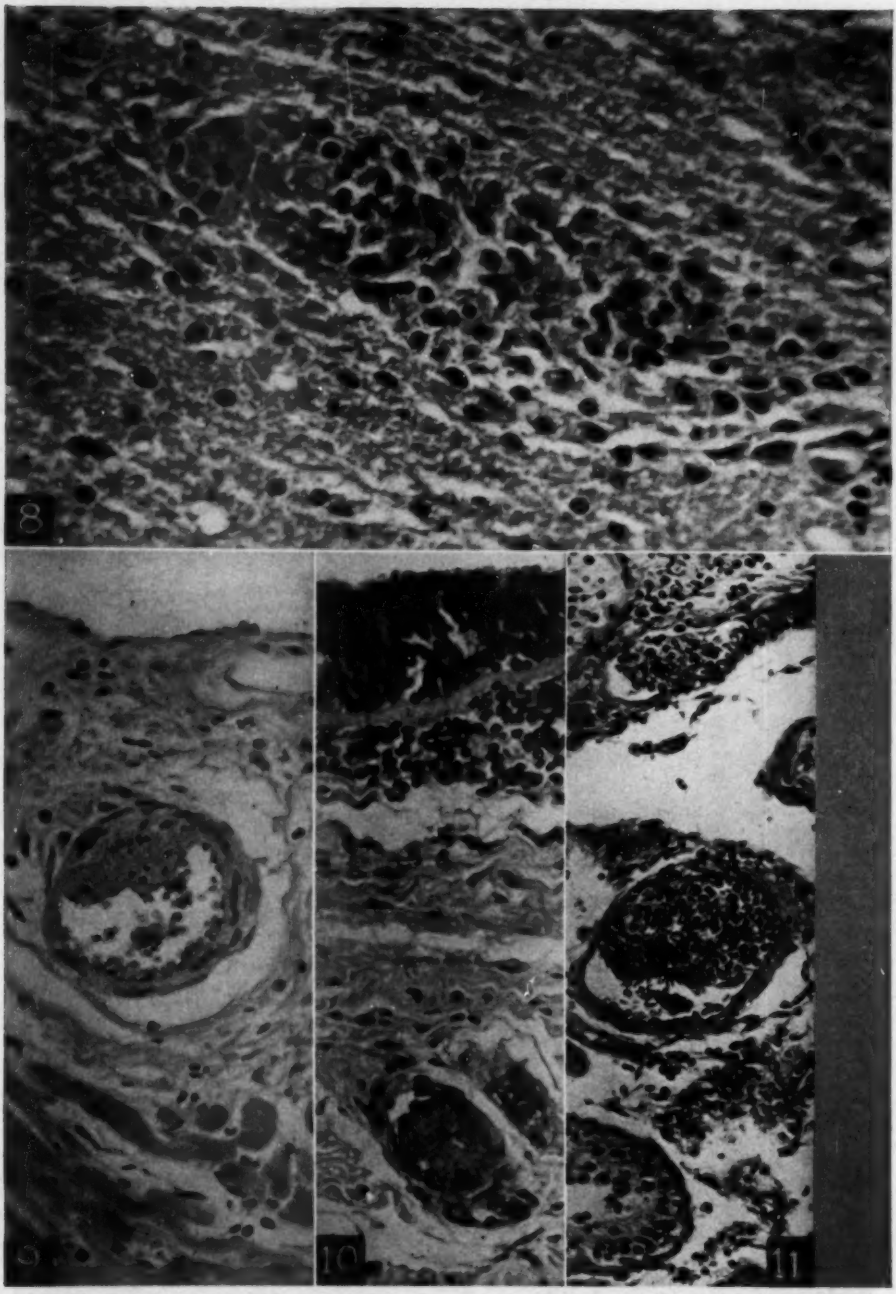

Fig. 8 (Case 4).—A large cerebral nodule showing its relation to a capillary in the most compact part; × 600.

Fig. 9 (Case 18).—Mural thrombus in subendocardial arteriole, without perivascular infiltration; × 350.

Fig. 10 (Case 18).—Granular thrombus in arteriole in submucosa of trachea and cellular infiltrate beneath basement membrane of mucosa; × 290.

Fig. 11. (Case 11).—Cellular thrombus in arteriole of testis; × 195.

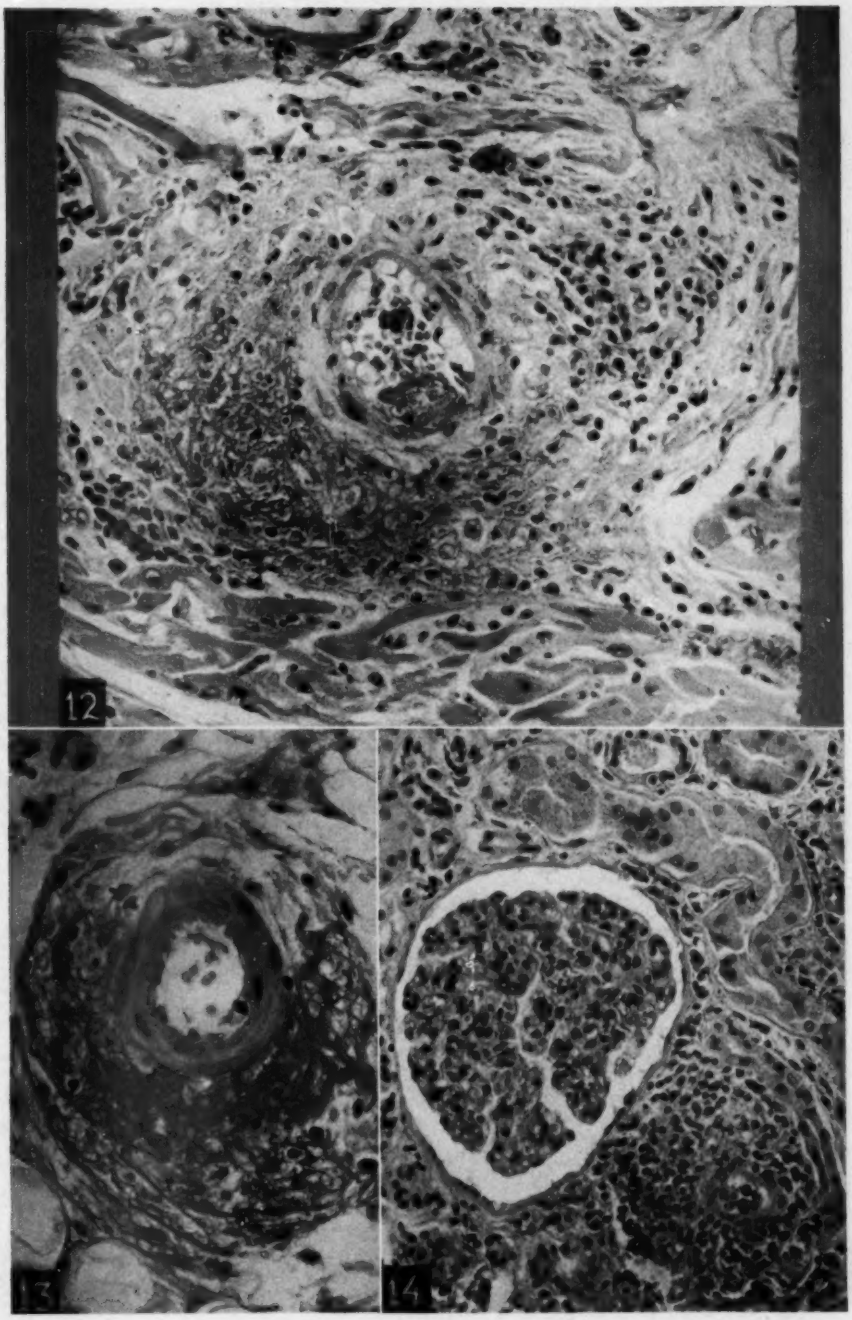

Fig. 12 (Case 22).—Arteriole in myocardium, with mural thrombus, fibrinoid degeneration in adventitia, and perivascular infiltration of many mononuclear cells and a few polymorphonuclear leucocytes; × 312.

Fig. 13 (Case 22).—Arteriole in epicardium, with endothelial swelling, fibrinoid degeneration in adventitia, but only slight perivascular cellular infiltration; × 370.

Fig. 14.—Periarteriolar nodule of mononuclear cells in renal cortex. The media and intima of the afferent arteriole are infiltrated and its lumen filled with exudative cells. There is moderate swelling of the glomerular endothelial cells and some degeneration of the tubular epithelium. The patient was a 32-year-old man who died after an illness of 13 days; × 252.

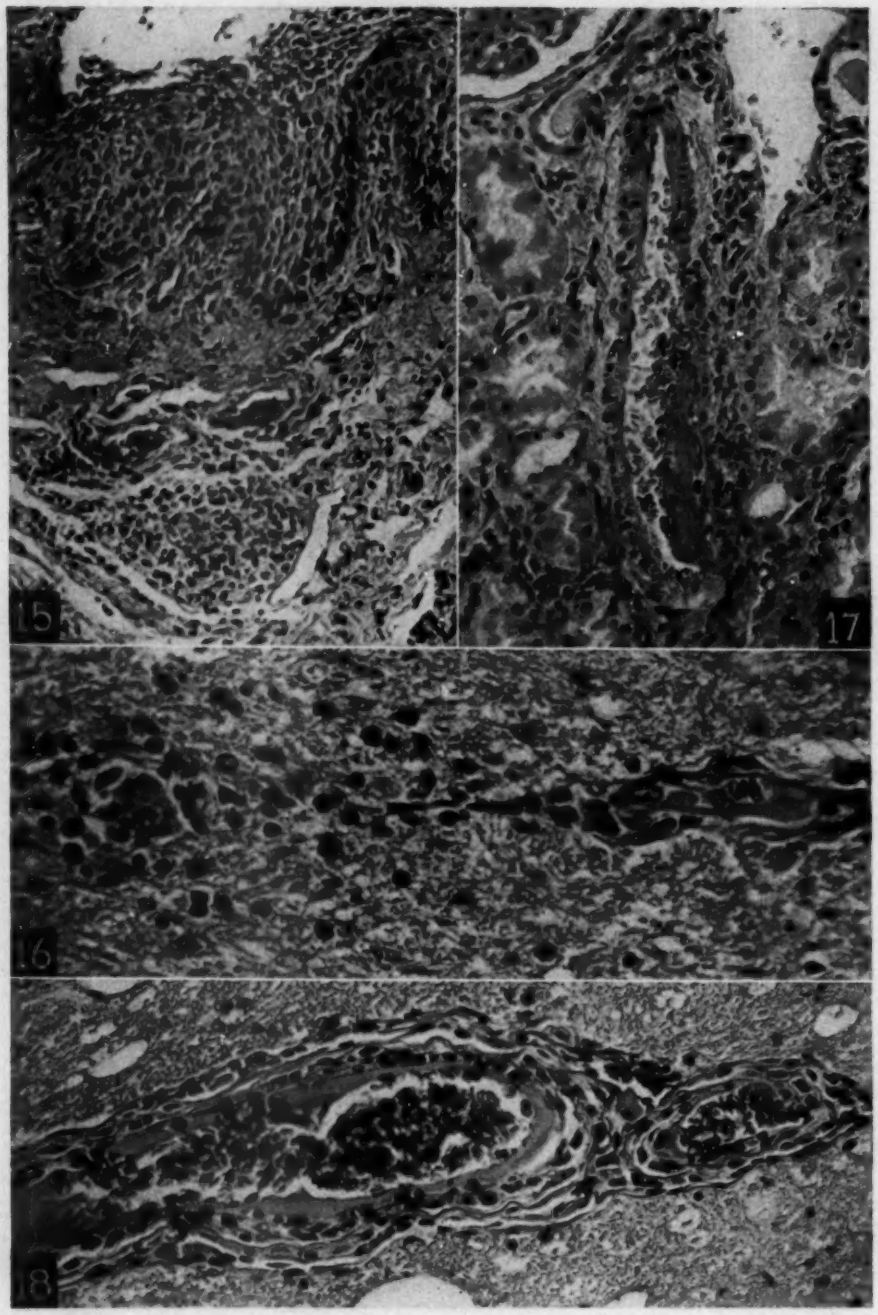

Fig. 15 (Case 26).—Arteriole in subepithelial tissue of palate showing necrosis and perivascular mononuclear cells; × 175.

Fig. 16 (Case 4).—Cerebral arteriole with perivascular accumulation of microglial cells; × 600.

Fig. 17.—Nonocclusive white mural thrombus in medium-sized artery of renal cortex. Endothelial cell proliferation is visible at one end, separating the thrombus from the free red blood cells in the lumen. Between the artery and an adjacent vein is an infiltrate of mononuclear cells with densely staining irregular nuclei. Cloudy swelling of proximal convoluted tubules with intraluminal granular precipitate is seen; × 252.

Fig. 18 (Case 3).—Moderate-sized artery in brain showing endothelial proliferation and swelling and mononuclear cells in adventitia; × 400.

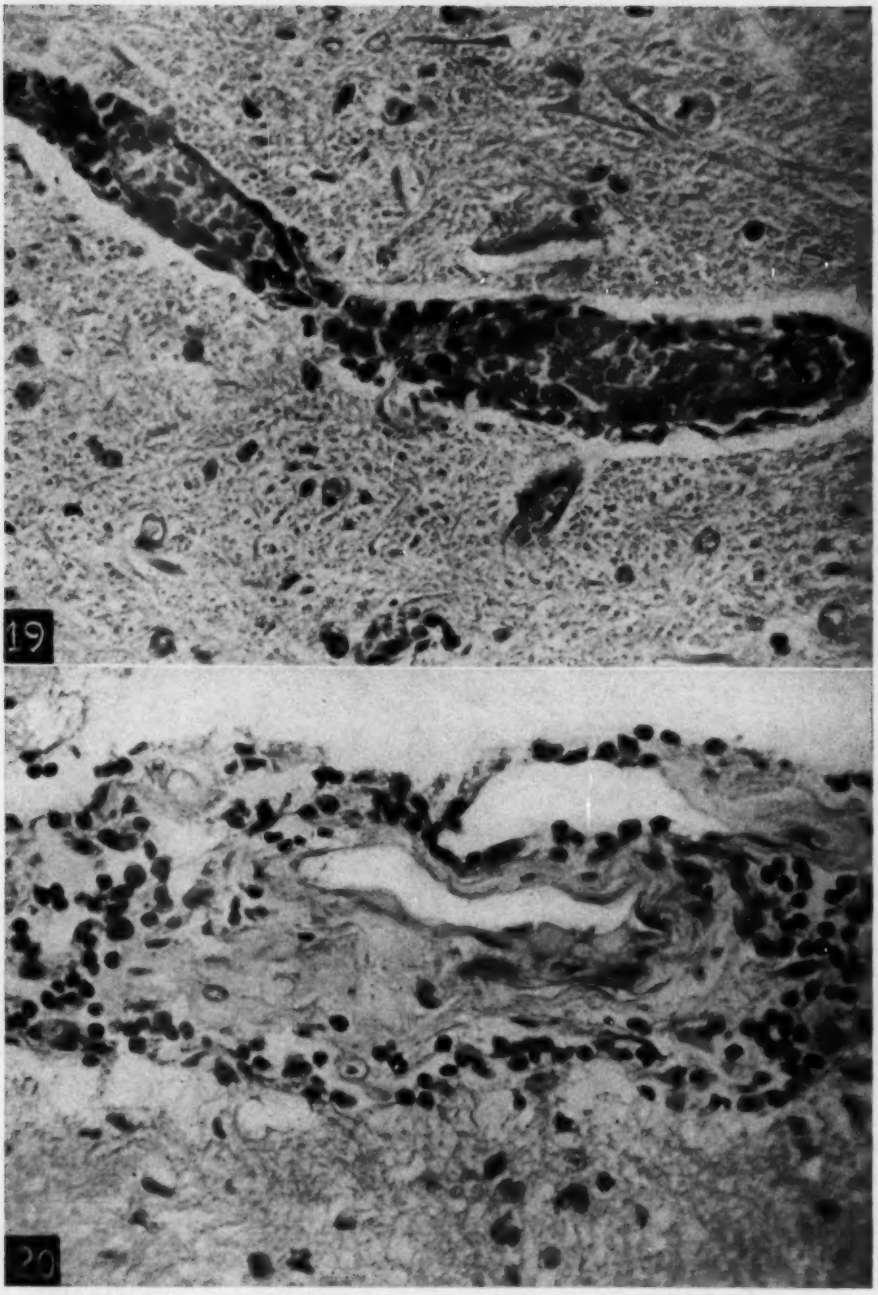

Fig. 19 (Case 33).—Moderate-sized cerebral artery showing details of perivascular mononuclear cells; × 600.

Fig. 20 (Case 4).—Cerebral vessel surrounded by mononuclear cells; × 600.

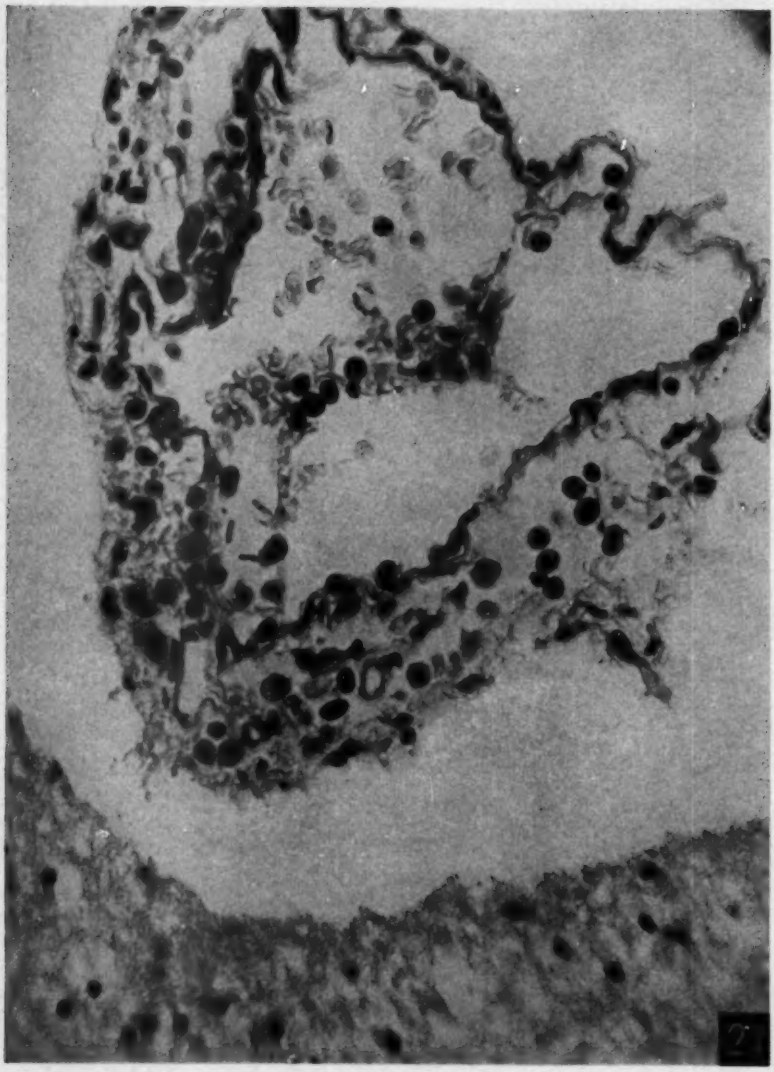

Fig. 21 (Case 16).—Perivascular mononuclear infiltration in the leptomeninges; × 600.

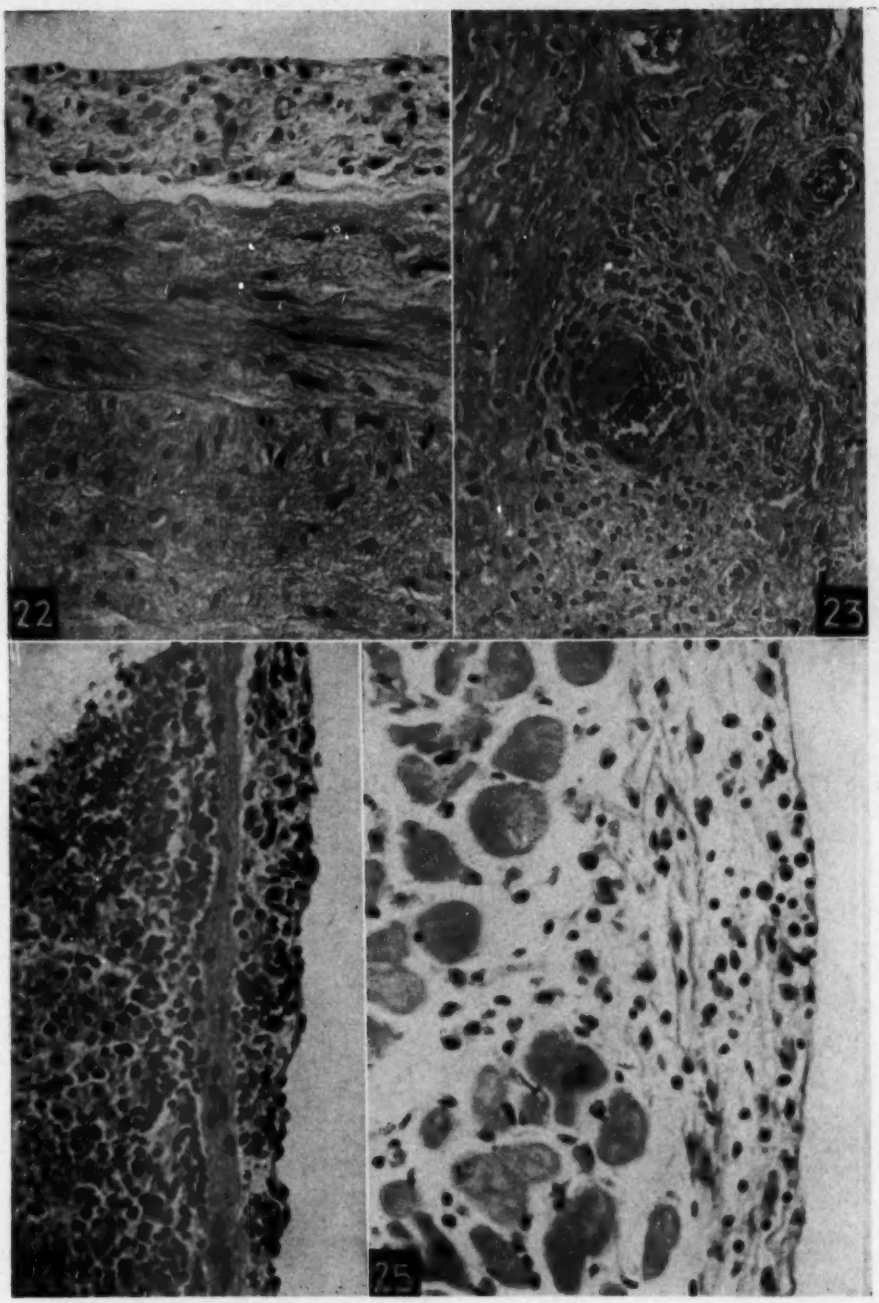

Fig. 22 (Case 23).—Mononuclear cellular infiltration into intima of aorta; × 312.

Fig. 23.—Perivenous nodule of mononuclear cells in renal pelvis. In the lumen is a white nonocclusive thrombus. Same patient as in Figure 14; × 252.

Fig. 24 (Case 13).—Intimal infiltration of mononuclear cells in splenic vein; × 270.

Fig. 25 (Case 23).—Diffuse infiltration of mononuclear cells in mural endocardium; × 312.

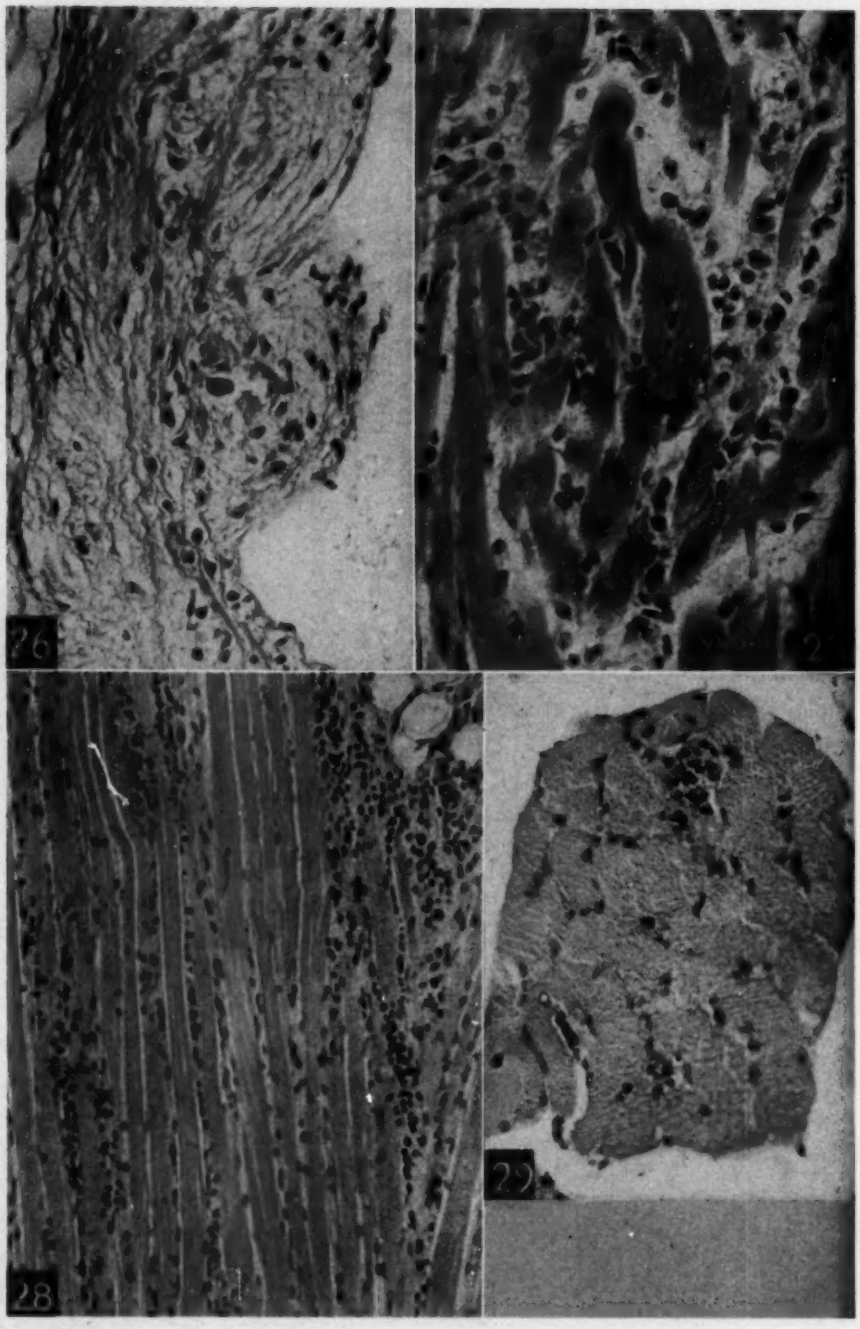

Fig. 26 (Case 20).—Point of attachment of base of mitral valve, with disruption of elastica, edema (mucoid), infiltration of mononuclear cells, and production of fibroblasts; × 312.

Fig. 27 (Case 23).—Acute interstitial myocarditis, with diffuse infiltration of mononuclear cells; × 312.

Fig. 28 (Case 18).—Acute interstitial myositis of tongue, with infiltration of mononuclear cells; × 155.

Fig. 29.—Small nodule of mononuclear cells in skeletal muscle attached to thyroid gland; × 234.

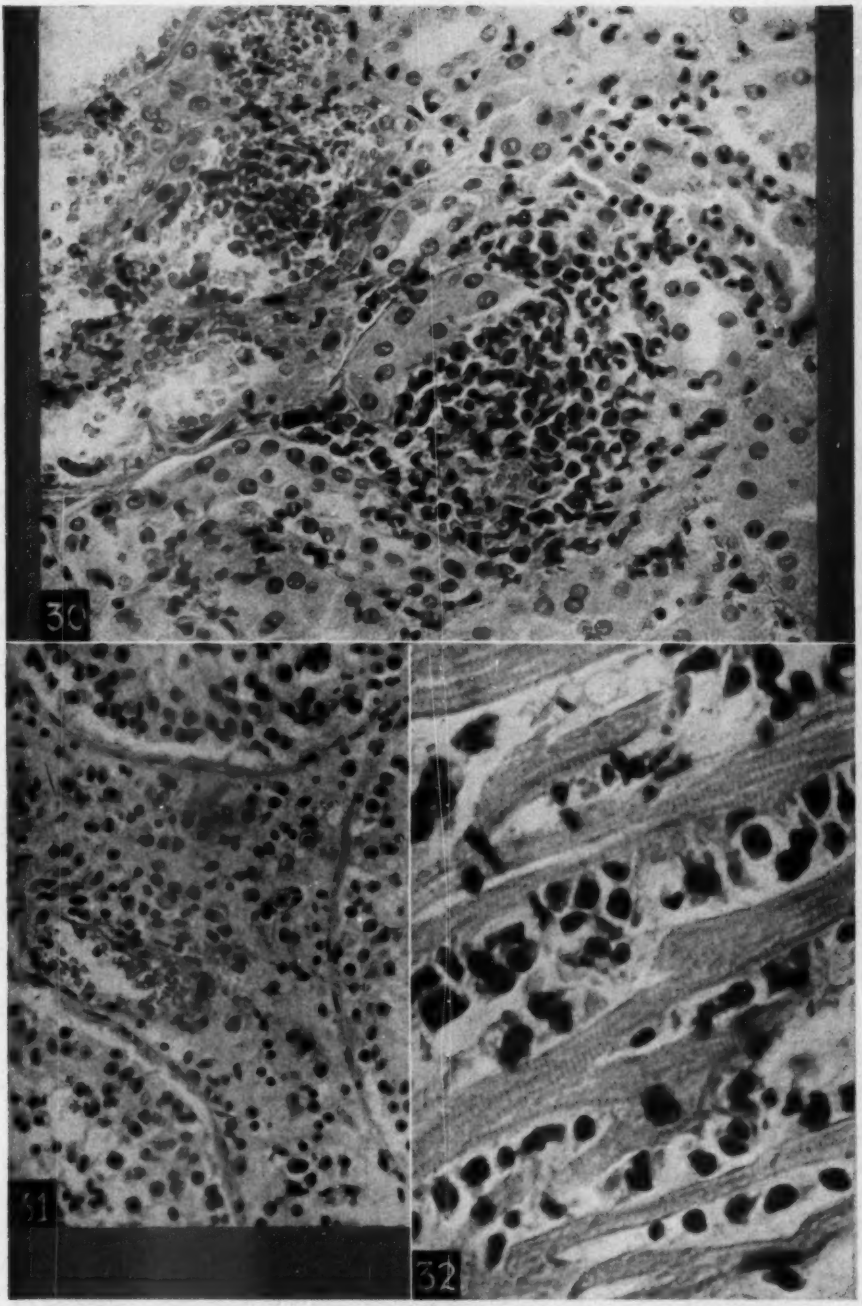

Fig. 30.—Mononuclear infiltrate in corticomedullary junction of kidney. Numerous cells have abundant basophilic cytoplasm and an eccentrically placed nucleus similar in chromatin pattern to that of plasma cells. Some of these are within the lumen of the small vessel, and the remainder are interstitial between the renal tubules. Numerous red blood cells are visible in the neighboring loops of Henle. The patient was a 30-year-old male, the duration of whose illness was not known; × 400.

Fig. 31.—Focal interstitial orchitis about a dilated capillary. The exudate is composed of large mononuclear cells, plasma cells, and lymphocytes; × 300.

Fig. 32 (Case 43).—Mononuclear cells forming interstitial exudate in acute myocarditis. The myocardial fibers are preserved; × 875.

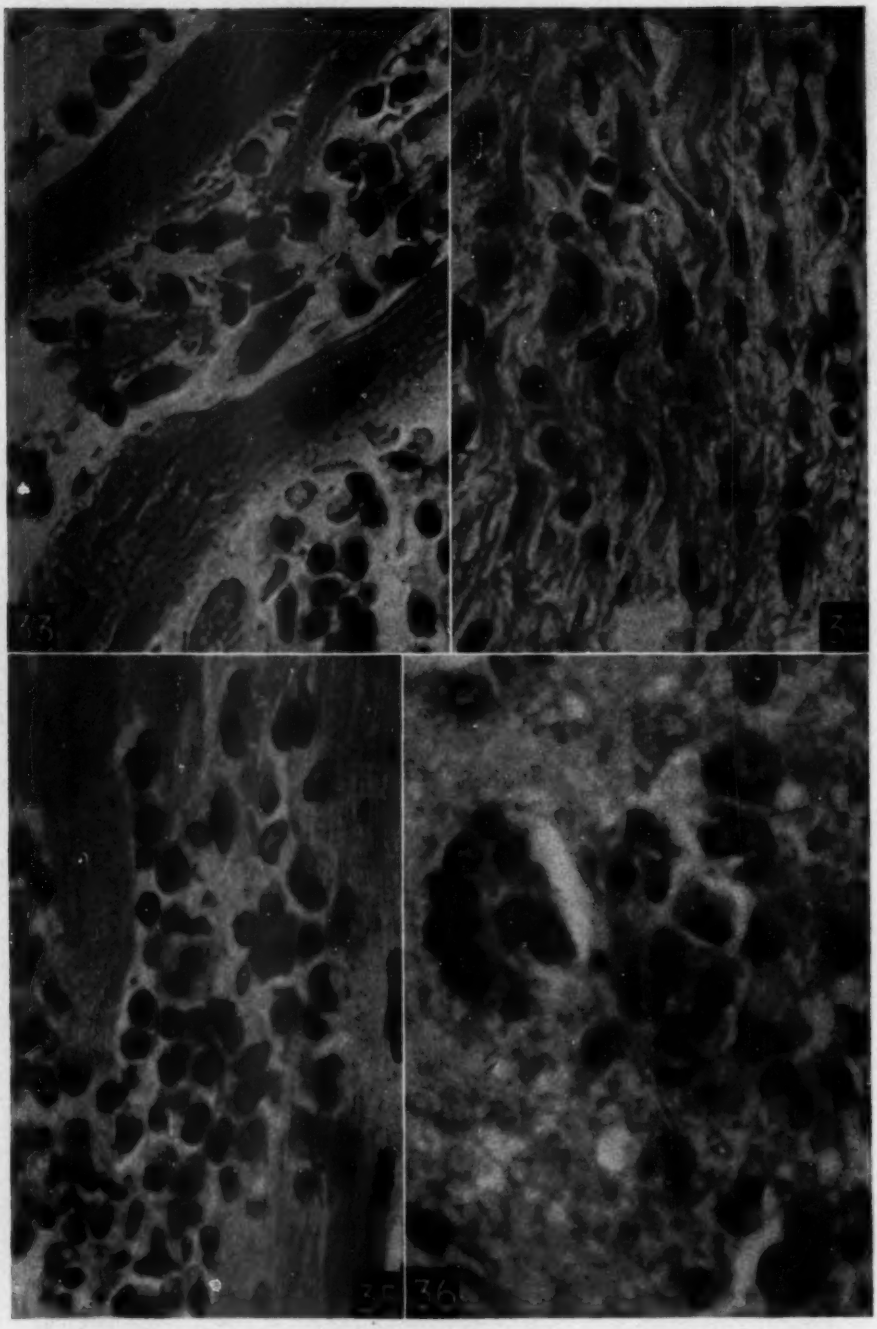

Fig. 33 (Case 43).—Acute myocarditis. Interstitial exudate of mononuclear cells, including several plasma cells; × 875.

Fig. 34 (Case 5).—Proliferation and swelling of mesenchymal cells in acute myocarditis; × 875.

Fig. 35 (Case 5).—Mononuclear cells in nodule in the muscle of the tongue showing their similarity to those in the myocardium, kidney, and brain; × 875.

Fig. 36 (Case 3).—Small cerebral nodule showing details of mononuclear cells; × 1,350.

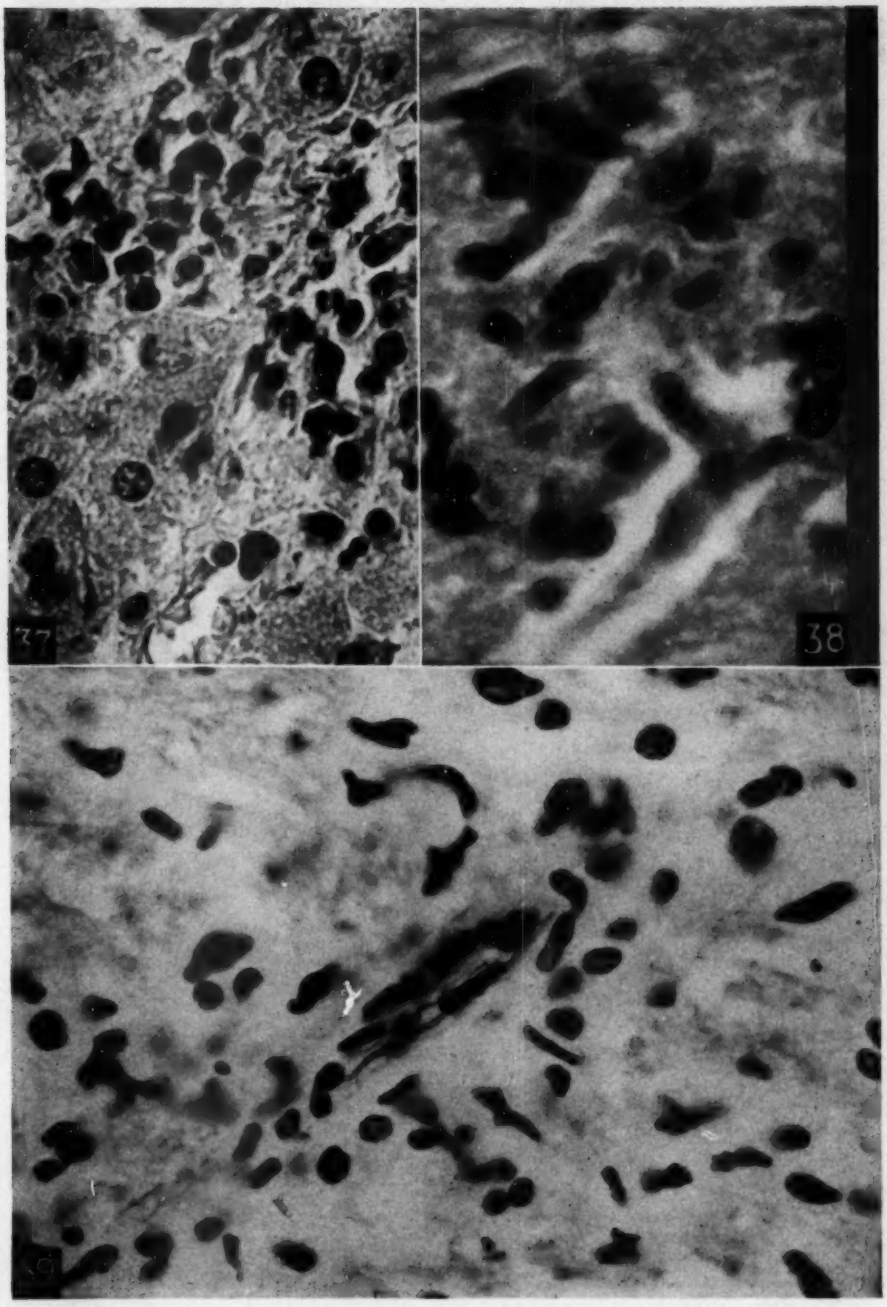

Fig. 37 (Case 43).—Collection of mononuclear cells in a small necrotic focus in the adrenal cortex; × 875.

Fig. 38 (Case 3).—A small cerebral nodule showing its pericapillary site; × 1,350.

Fig. 39.—Wilder's reticulin stain of a moderate-sized nodule showing persistence of reticulin fibers; the central mass probably represents the central capillary; × 1,320.

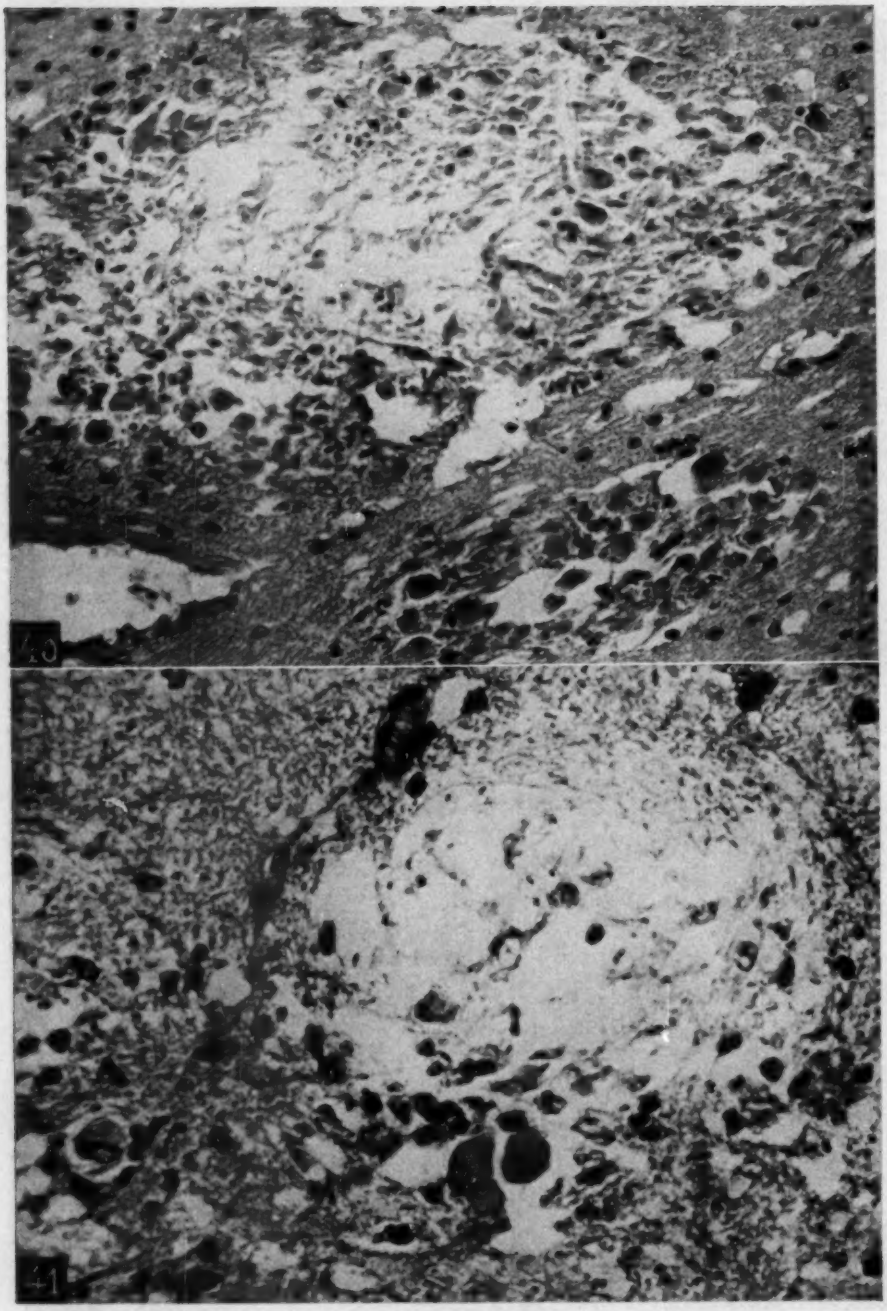

Fig. 40 (Case 30).—A focus of swollen axons and disruption of white matter (Lillie's microinfarct). Note variations in size from normal axons to huge bodies; × 340.

Fig. 41 (Case 30).—A small focus of disrupted white matter with a few swollen axons; × 600.

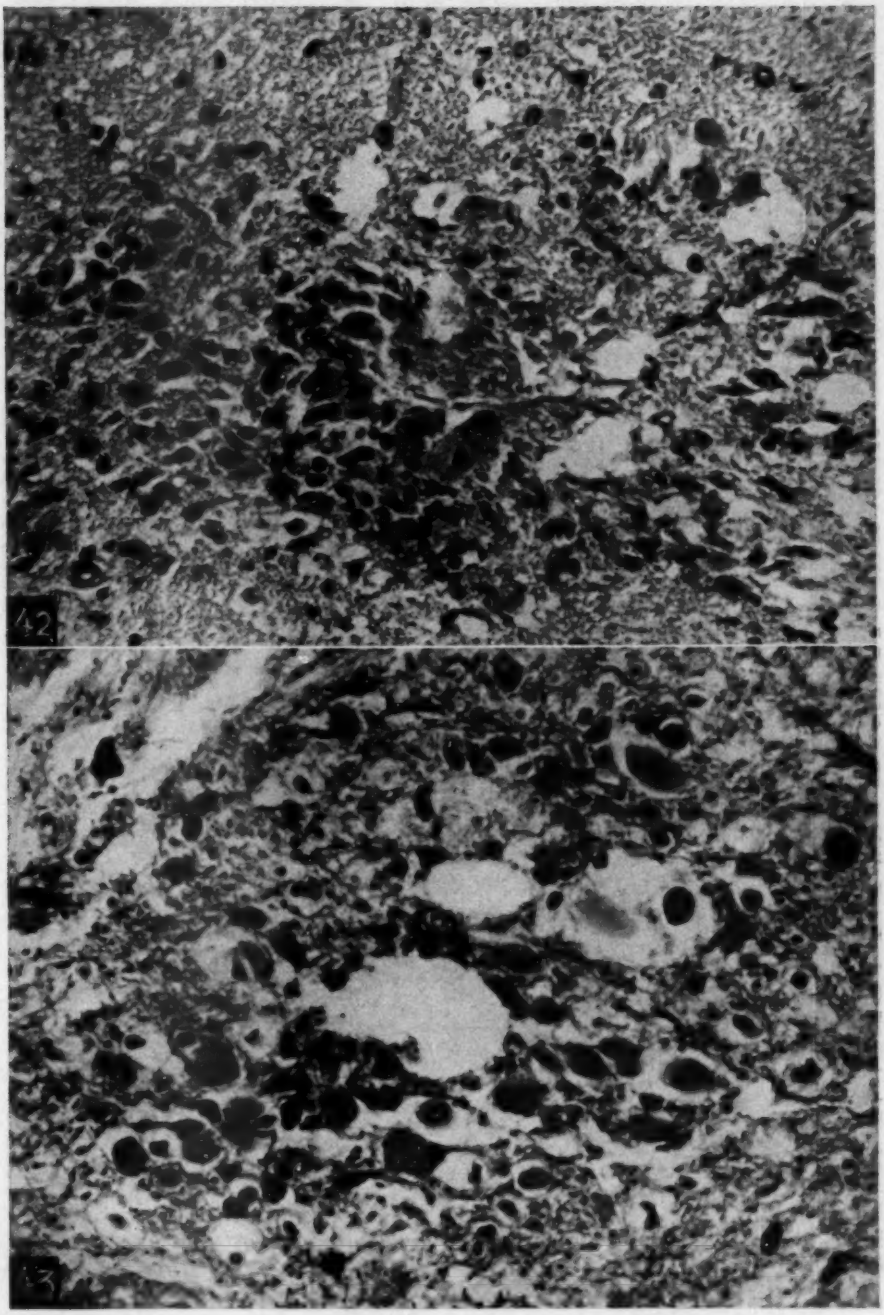

Fig. 42 (Case 30).—A lesion that combines the features of a nodule and a "microinfarct." Some of the swollen axons are cut tangentially, showing that they are cylindrical; × 320.

Fig. 43 (Case 33).—A small focus of swollen axons that shows particularly well the variation in size of the axis cylinders; × 750.

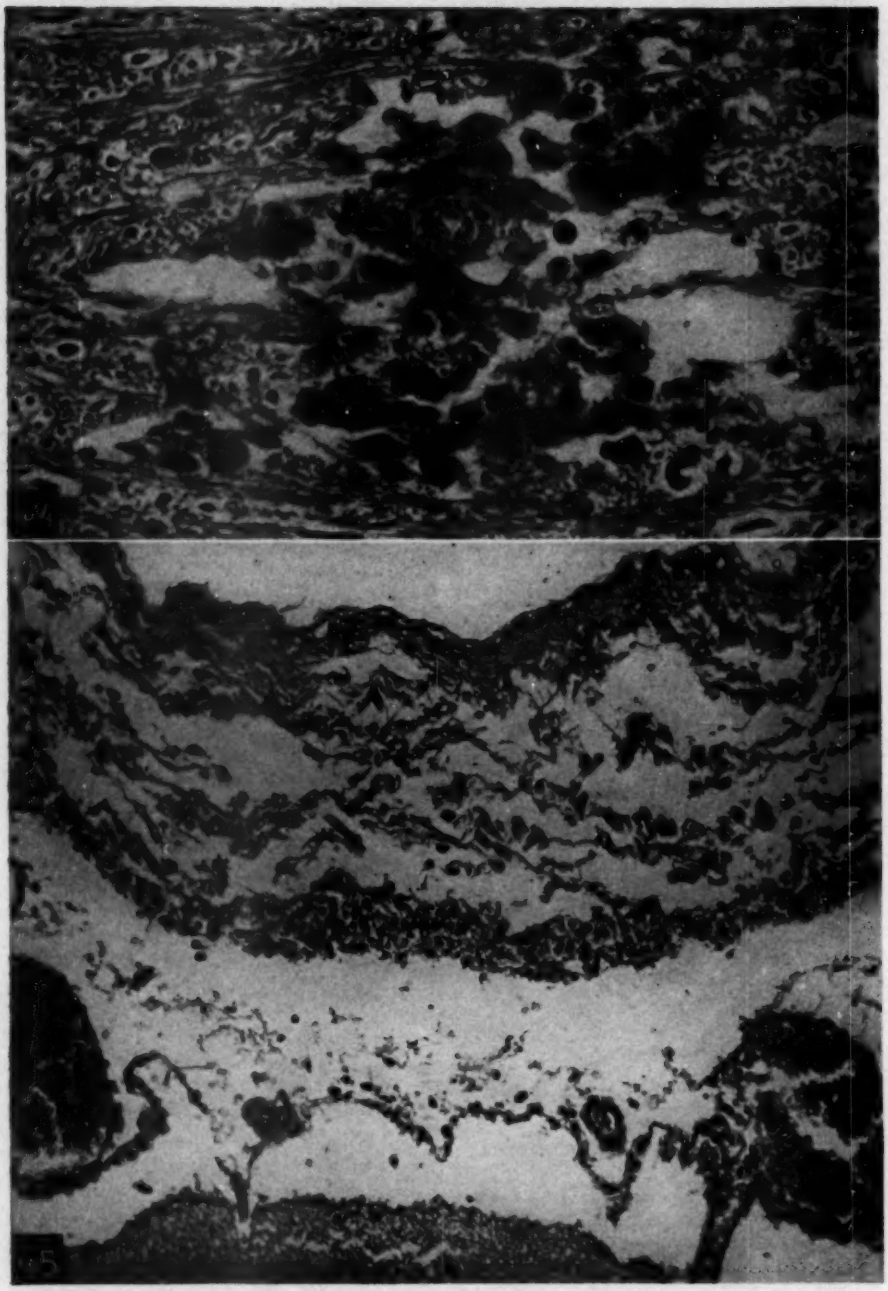

Fig. 44 (Case 30).—Holmes's silver impregnation of a focus of swollen axons in the basalis portis. The involved fibers run perpendicularly to the section. The swollen axons are not as densely argentophilic as normal; × 750.

Fig. 45 (Case 33).—Diffuse infiltration of mononuclear cells in the pia arachnoid; × 170.

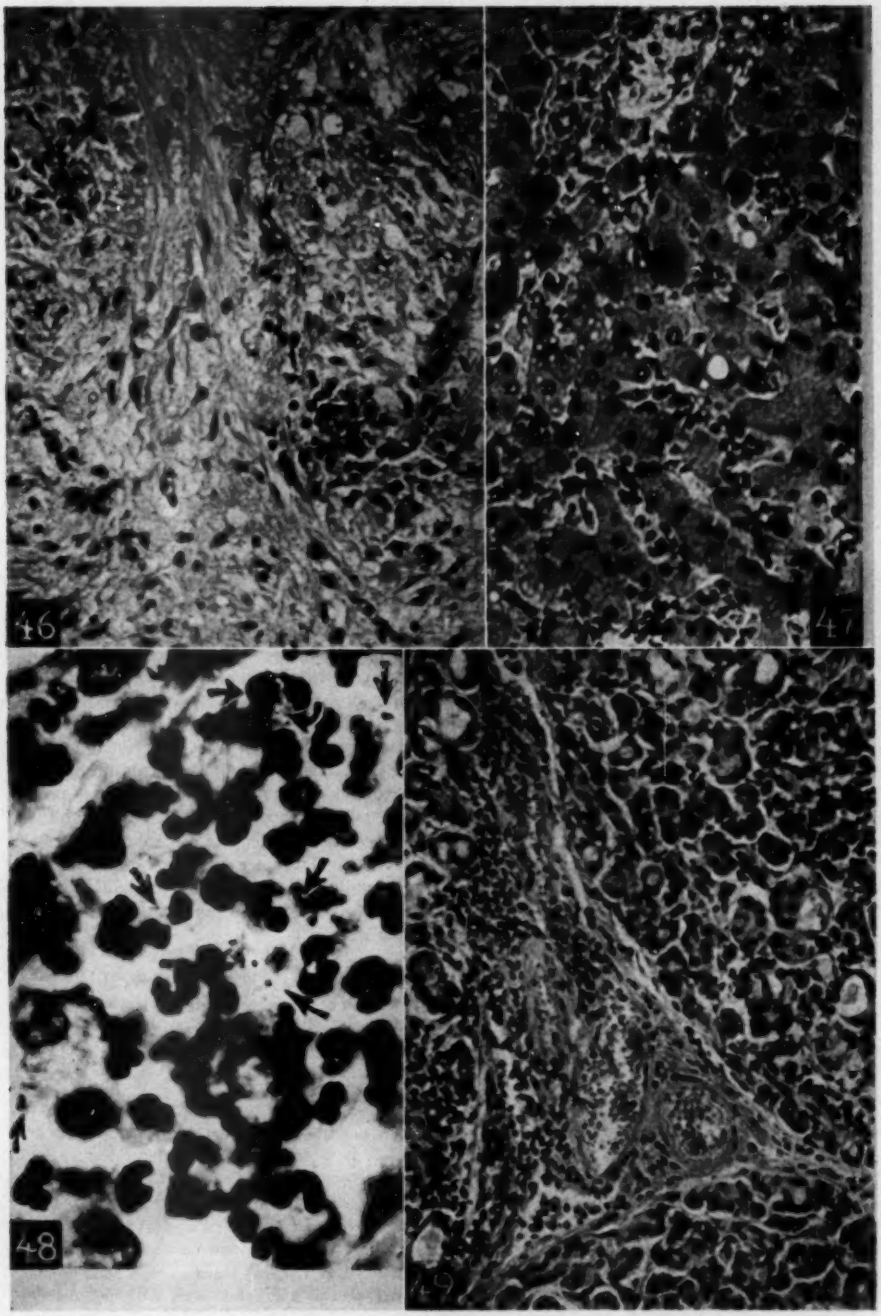

Fig. 46 (Case 23).—Nodules in posterior lobe of pituitary identical with those in the brain and spinal cord; × 250.

Fig. 47 (Case 11).—Hyperplasia of sinusoidal endothelium of the liver and infiltration of mononuclear cells; × 250.

Fig. 48.—Lung, alveolar exudate showing many rickettsia-like bodies in cytoplasm of polymorphonuclear leucocytes. Nyka stain; × 1,450.

Fig. 49 (Case 13).—Pancreas, infiltration of mononuclear cells in the interlobular septum, dilatation of acini and inspissation of secretion; × 205.

Books

Stress Incontinence in the Female. By John C. Ullery, M.D., F.A.C.S., F.I.C.S., Obstetrician and Gynecologist, Pennsylvania Hospital: Assistant Professor of Obstetrics and Gynecology, Jefferson Medical College of Philadelphia; Associate Professor in Gynecology and Obstetrics, The Medico-Chirurgical College, Graduate School of Medicine, University of Pennsylvania; Chief of Obstetrics and Gynecology, Philadelphia General Hospital; Chief of Obstetrics and Gynecology, Delaware County Hospital, Drexel Hill, Pa. Price, $6.75. Pp. 149, with 82 illustrations. Grune & Stratton, Inc., 381 Fourth Ave, New York 16, 1953.

Surgery of the Pancreas. By Richard B. Cattell, M.D., Surgeon, the Lahey Clinic; New England Baptist Hospital; New England Deaconess Hospital, Boston, and Kenneth W. Warren, M.D., Surgeon, the Lahey Clinic; New England Baptist Hospital; New England Deaconess Hospital, Boston. Price, $10.00. Pp. 374, with 100 figures. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia, 1953.

This monograph is based upon the authors' experiences in the management of over one thousand patients suffering from pancreatic disease. The anatomy and physiology, as well as the embryology and histology, are briefly described. There is an excellent review of the blood supply of the pancreas. The authors discuss, in turn, congenital malformations, acute and chronic pancreatitis, cysts, injuries, islet cell adnomas, and carcinoma of the pancreas. The chapters on acute and chronic pancreatitis are exceptionally well done. Illustrations of the clinical course of each disease are given in brief but complete case histories. Even though emphasis throughout is given to the clinical aspects of each condition, the discussions of etiology and pathology are by no means slighted. The value of the different surgical procedures used in the treatment of each type of pancreatic disease is critically appraised. An outline of the technique of total pancreatectomy, included in the chapter on carcinoma of the pancreas, is the only extensive discussion of operative technique. In the final chapter the place of total pancreatectomy in the treatment of pancreatic disease is carefully evaluated. A lengthy bibliography documents each subject. The book is highly recommended not only to surgeons for detailed study but also to all physicians interested in pancreatic disease.

Handbook of Tropical Dermatology and Medical Mycology. Edited by R. D. G. Ph. Simons, Senior Lecturer at the Dermatological Clinic of the University of Leyden; Dermatologist in Charge at the Civilian Hospital, Amsterdam. Price,. $15.00. Pp. 845, with 587 illustrations. Elsevier Press, Inc., 402 Lovett Blvd., Houston 6; 300 Park Ave., New York, 1952.

This work represents a cooperative effort by a group of eighty contributors from various parts of the world to present information about diseases of the skin that are encountered in tropical areas. Volume 1 of this two-volume handbook is introduced by a general survey by Simons, in which the editor writes interestingly of the history of tropical dermatology, attempts to simplify the confused terminology, and discusses briefly the less important dermatoses. The following chapters are devoted to dermatoses in children; pigmentary disorders; depigmentations; diseases due to protozoa, spirochetes, bacteria, cocci, rickettsiae, and viruses; the miliaria group, and tropical acne. Even though these are discussed as tropical diseases, the book takes on added importance for us because many of these dermatoses are being seen with increasing frequency in the United States. Some occur in individuals whose travels have taken them to tropical areas. In addition, the climatic and social-economic conditions that endow many of these diseases with their special "tropical qualities" are at times duplicated in parts of our own country. A study of this book is recommended as a means of alerting us to recognize variations of dermatoses that might at times escape early detection.

While the emphasis is chiefly on diagnosis and treatment, for many of the diseases there is a discussion of the pathology as well. The book is profusely illustrated, and there are many excellent black and white clinical photographs and photomicrographs. Each chapter contains numerous references for those desiring more extensive pertinent information.

The Mechanisms of Disease: A Study of the Autonomic Nervous System, the Endocrine System and the Electrolytes in Their Relationship to Clinical Medicine. By Joseph Stambul, M.D., Chief of Cardiovascular Department, Albert Einstein Medical Center (Eastern Division), Philadelphia. Foreword by George Morris Piersol, M.D., Professor of Medicine, Graduate School of Medicine, University of Pennsylvania, Philadelphia. Price $15.00. Pp. 746. Froben Press, Inc., 1776 Broadway, New York 19, 1952.

The author of this book attempts to lead the way to a better understanding of disease by reducing the processes which underlie disease to changes in individual cells. In other words, Virchow's cellular pathology is to be translated to a cellular pathologic physiology. In order to accomplish this end the author "collated into a single volume . . . facts widely scattered throughout the medical and allied literature . . . for quick perusal and understanding" (Preface). In keeping with this statement of purpose, various subjects have been reviewed in abstract form in ten sections and forty-three chapters. Cell structure, cell metabolism, and cell membrane permeability are the subjects of Section 1. Liver is treated in the three chapters of Section 2; fat in Section 3; the reticulendothelial cells, cholesterol, and hypercholesterolemia in Sections 4 and 5; heart and skeletal muscles in Section 6; capillaries in Section 7; endocrine glands, with schizophrenia included, in Section 8; local inflammation and bacterial diseases in Section 9, and the action of drugs in the concluding section.

The information presented consists of abstracts of a fairly voluminous bibliography listed on forty-three pages, each page containing about forty items. It seems that the collection of data must have been reduced considerably about 1945. In a survey of the first ten pages of the bibliography, only nine titles were dated after that year. A striking example of neglect of more recent literature is that several quotations from Boyd's "Pathology of Internal Diseases" date back to the 1935 edition. The author of the book is credited with only one entry relating to unpublished experiments of 1941.

The book is essentially a compilation of data loosely put together. There is little evidence of a critical evaluation, which is essential if a work of this type is to be more than a gathering of abstracts. Even the comments and summaries at the ends of the chapters are again collections of abstracts.

The book abounds in controversial statements, which may be quotations taken out of context.

The volume represents a commendable effort on the part of the author to gather a great deal of assorted information for his enlightenment and an equally commendable desire to share it with others. Whether publication of such material in book form was justified is open to serious question.

The Physiology and Pathology of Hemostasis. By Armand J. Quick, Ph.D., M.D., Professor of Biochemistry, Marquette University School of Medicine. Price, $4.00. Pp. 188, with 18 illustrations and 21 tables. Lea & Febiger, 600 S. Washington Sq., Philadelphia 5, 1951.

The author has successfully presented the problem of hemostasis with a clarity rarely achieved in this complex field. The subject is introduced by a brief historical sketch which enables the reader to gain a clear perspective of the past and present problems of hemostasis. The various components known to be essential for blood coagulation are discussed individually but always in relation to the total mechanism of coagulation. After introducing the subject, Dr. Quick formulates a theory for hemostasis which considers not only blood coagulation but also other factors important in hemostasis, such as clot retraction, endothelial changes, and serotonin.

In the remaining chapters of his book, the author integrates basic physiology with the clinical problems of hemorrhagic diseases and venous thrombosis, and the surgical management of patients with bleeding tendencies.

The book is then concluded with a discussion and a description of laboratory methods used in the diagnosis and treatment of hemorrhagic and thrombotic disease.

The result is a book which offers to the physician and student a concise well-written account of the basic physiological and clinical problems in the field of hemostasis.

Advances In Veterinary Science. Edited by C. A. Brandly, University of Wisconsin, and E. L. Jungherr, University of Connecticut. Volume I. Advisory Board: Sir Thomas Dalling, P. J. DuToit, W. Frei, W. A. Hagan, A. Hjarre, C. A. Mitchell, and B. T. Simms. Price, $9.00. Pp. 431, with charts, tables, and illustrations. Academic Press Inc., 125 E. 23rd St., New York 10, 1953.

Cerebral Changes Following Electrically Induced Convulsions: An Experimental Study on Cats. By Hans Hartelius. Supplement 77, Acta psychiatrica et neurologica scandinavica. Price, 20 Swedish crowns. Pp. 128, with 24 figures and 17 tables. Ejnar Munksgaard, Forlag, Nørregarde 6, Copenhagen K., 1952.

Major Metabolic Fuels: Brookhaven Symposia in Biology No. 5, September, 1952. Price, $1.35. Pp. 234, with tables, charts, and diagrams. Brookhaven National Laboratory, Upton, L. I., N. Y., 1953.

The Book of Health: A Medical Encyclopedia for Everyone. Compiled and edited by Randolph Lee Clark Jr., B.S., M.D., M.Sc. (Surgery), Houston, Texas; Director and Surgeon-in-Chief, The University of Texas M.D. Anderson Hospital for Cancer Research; Professor of Surgery, University of Texas Post-Graduate School of Medicine; Fellow of the American College of Surgeons; National Consultant in Surgery, United States Air Force, and Russell W. Cumley, B.A., M.A., Ph.D., Houston, Texas, Director of Publications, The University of Texas M.D. Anderson Hospital for Cancer Research; Professor of Medical Journalism. University of Texas Post-Graduate School of Medicine. Price, $10.00. Pp. 768, with 1,400 illustrations. Elsevier Press Inc., 402 Lovett Blvd., Houston 6, Texas; 155 E. 82nd St., New York 28, 1953.

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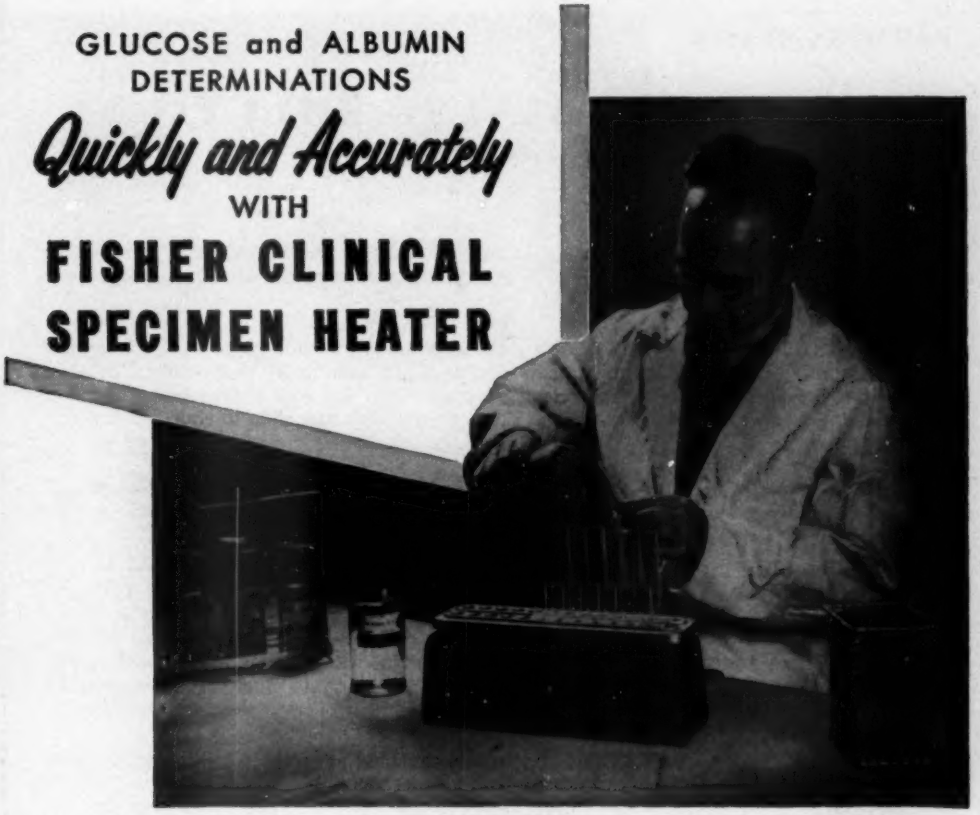

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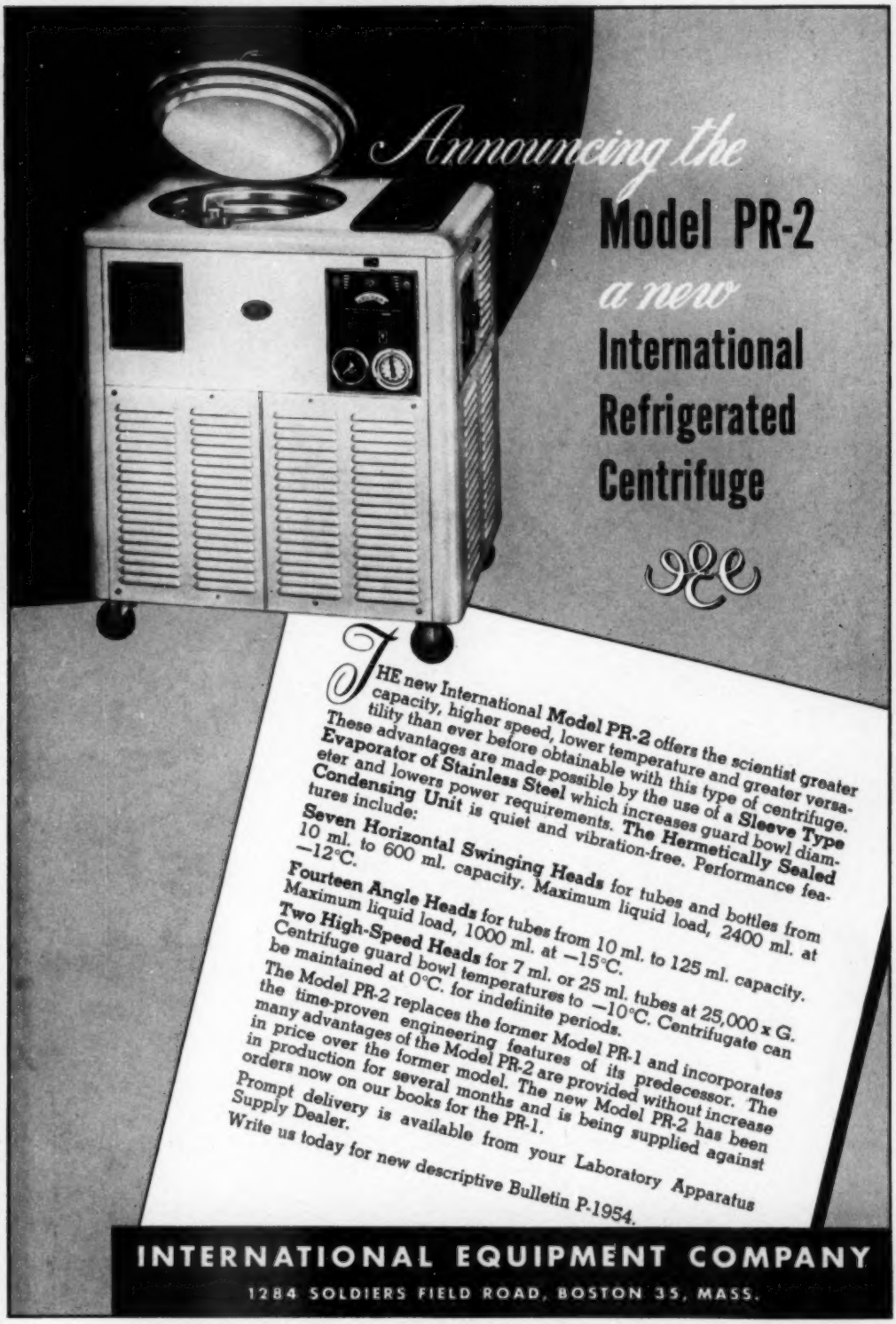

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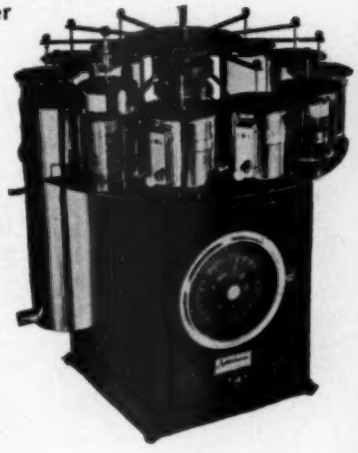

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